AMERICAN

Industrial Hygiene

ASSOCIATION

Journal

VOLUME 19, NUMBER 3

JUNE 1958

Pangborn Dust Control

gives you healthier, happier employees!

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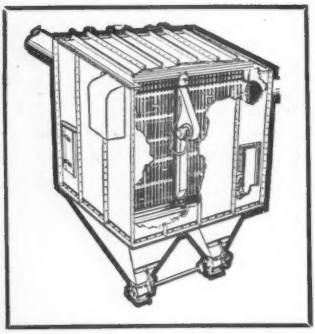
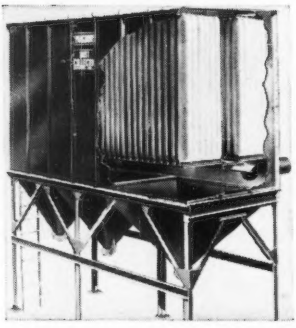
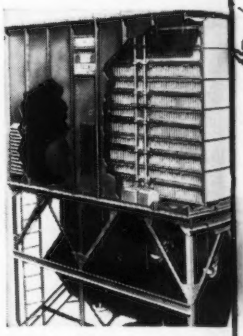


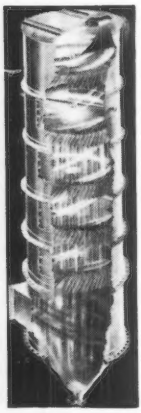
For automatic, *continuous* operation, the Pangborn Self-Cleaning Collector — combining the proved efficiency of the cloth screen collector with continuous operation, uniform air flow.



Also for dry dusts, the Pangborn Cloth Bag Collector—an efficient, economical method of dust control. Its simplified design and construction involves a minimum of parts. Easily inspected and maintained.



For fine, dry dusts, the Pangborn Screen Collector—accomplishes recovery at low cost; easily tained and gives maximum area for the space available.



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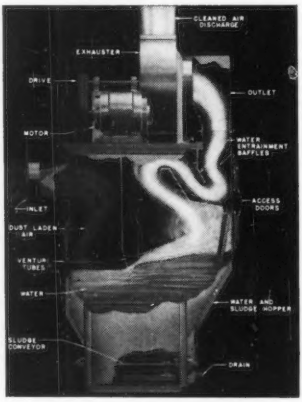
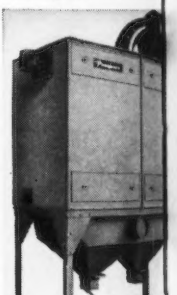


The Pangborn Ventrijet Wet Collector—also for dusts that a dry type collector cannot handle. A compact, self-contained unit with low head-room. Inexpensive to maintain and operate, its ease and economy of installation recommend its use in many cases.



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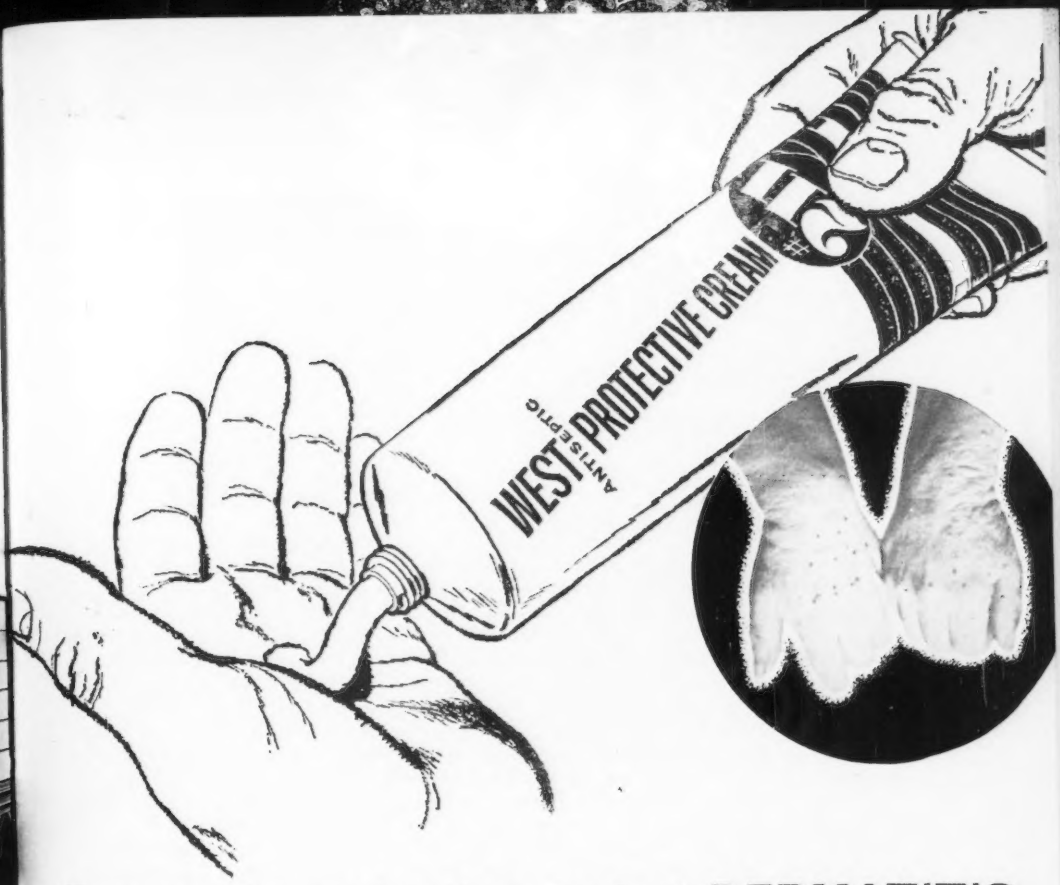
NOTE: Bulletins are available on all Pangborn Dust Collectors. For your copy or more details, write PANGBORN CORP., 4500 Pangborn Blvd., Hagerstown, Md. *Manufacturers of Dust Control and Blast Cleaning Equipment.*

Pangborn CONTROLS DUS



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President's Page

Professional status is a matter of special interest to industrial hygienists because we are not well known to the public or even to some professional groups. This lack of recognition is discomforting to many of us. More importantly, it may at times hinder acceptance of us and our work. It is awkward enough to have to explain our work to friends and acquaintances; it is worse to have to explain to a plant manager before viewing his plant operations and perhaps recommending changes that may increase his operating costs. In pensive moments some may have wondered whether industrial hygiene is indeed a true profession.

A clear and thoughtful discussion of professional status was given by Vannevar Bush in the 11th Martin memorial lecture delivered before the clinical congress of the American College of Surgeons in San Francisco on October 8, 1956 and reprinted in *SCIENCE, 125,* 49 (1957). In defining a profession Dr. Bush gave five criteria which I paraphrase for brevity. First, members of it (the profession) possess special knowledge acquired by long study. Second, it is a loose, voluntary grouping of independent members. Third, it has to a degree a kind of symbolism and ritual of its own. Fourth, it often has a means for maintaining standards and for disciplining violators of its own code. And fifth—a primary characteristic, Dr. Bush says—a profession is composed of members who minister to the people—minister in the sense of advising, in their special field, with confidence, authority and dignity.

Judgment of industrial hygiene by these criteria is simple. On points one, two and three there is no doubt. On point four, we have had certain minimum requirements for membership in the American Industrial Hygiene Association for nineteen years. Further progress is now being made by joint AIHA-ACGIH development of certification standards and by AIHA's Committee on Ethics.

At first thought we may seem to be lacking with respect to point five. But not so. To minister means to attend or to do things needful or helpful. Industrial Hygienists do not often attend people, but they do indeed do things needful or helpful. The toxicologist assaying the toxicity of a new industrial chemical is certainly ministering, though indirectly, to the people. The industrial hygiene work of the engineer, the chemist, the physician and the physicist represents equally well a ministration to people. No matter that our function is prevention of health impairment rather than amelioration or cure. Industrial hygiene is ministering. It is professional.

Kenneth W. Nelson

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Cummings Memorial Lecture*

MAJOR GENERAL JAMES P. COONEY, MC

Deputy Surgeon General, United States Army

Major General James P. Cooney, MC

WHEN YOU did me the honor to ask me to deliver the Cummings Memorial Lecture, I gave considerable thought to the selection of a topic. I believe I have chosen an appropriate subject for this lecture—Man's Relationship to Man.

During the past several decades, profound changes have taken place in American society. These changes are a result of participation in two global conflicts, the fantastic improvements in the means of travel and communication, a large displacement of our rural population into extensive urban communities, industrialization and redevelopment of sections of our country formerly predominantly agricultural, and the extension of our educational system to every nook and cranny of our country. We have assumed the role of leadership of the remaining free world in an attempt to stem the tide of communism and, as a result, have become the target of criticism of both ally and the communist camp. Our people have become increasingly sensitive to criticism both at home and abroad, especially when that criticism is aimed at defects in our social structure.

A tremendous revolution has occurred in American industry. The introduction of semiautomatic machines, assembly line techniques and increasing automation has reduced the importance of the skilled craftsman. The net product of our economy has made more of the material goods available to our people and made competition for the domestic and foreign market more intensive. The unionization of mass-production industries and the passage of legislation favorable to Labor has increased respect for the common working man. Finally, the old-time rugged individualist, the captain of industry who had complete domination of his particular enterprise, has been replaced by a team of management experts. This management team is composed of specialists in finance, tax and legal problems, production engineering, sales and advertising, research and analysis, and advisors on personnel problems. Irrespective of the talent and professional competence of the individuals composing the top echelons of any organization, the effectiveness of that organization is only as good as the interpersonal relations existing among the people in the organization.

The most striking change in our century is the change that has taken place in our attitude towards human nature and human beings. Newer knowledge in psychology has given us better insight into human nature, human behavior, human relations, and human development. Coincidental with the development of knowledge in human behavior, the machine age has made a tremendous impact upon the social, economic, and political system of our country. Now, all of us find ourselves participating in world affairs, sensitive about our rights and duties as citizens of the United States and increasingly conscious that we cannot ignore our neighbor or our fellow worker.

All the tools available to any organization are, in the final analysis, wielded by the individual. For that reason, I have elected to talk to you about the human being. Despite academic definitions, I am covinced that the most serious problems encountered in any organization, hospital, factory, supermarket, school, church or government are in the field of interpersonal relations, or those interactions which take place between individuals and other individuals or groups. Most of the friction that develops among people arises from a feeling of inferiority on the part of an individual, his vanity, false pride

* American Industrial Hygiene Association, Atlantic City, New Jersey, April 23, 1958.

and an unwillingness to subordinate himself to someone else. As a consequence, to accomplish his purpose he may resort to the desire to "throw his weight around." Happy is the man who has the confidence of his own ability to make his mark in another area and make room for his fellow man when the occasion arises.

My concept of good interpersonal relations implies unity and cooperation among people. Essentially, to achieve these, nothing more is necessary than the practice of good will and common sense to prevent misunderstanding. There are numerous sources of misunderstanding. Often-times, misunderstanding results from the different positions held by two individuals. For a supervisor and an employee, the rules, objectives and the regulations of an organization have a different meaning. Individual attitudes of people cause them to view problems differently. What is viewed as regimentation by one person may be regarded as system and good order by another. We frequently misjudge people because we are unaware of their specific needs, or because we attempt to apply our own standards of right and wrong. One individual strives for promotion because of the prestige involved, another because of the additional pay and a third, simply because he does not want to be passed over.

For each need, there is a different substitute, but the same substitute may be provided for a group of individuals. Frustrated people may be misunderstood because the problems of their job are not considered. By properly evaluating the factors involved, the problems of the job may be eliminated or reduced. By understanding the reasons for human behavior people change their attitudes. This, then, suggests that there is something we can do to reduce misunderstanding. As soon as we recognize this fact many problems become soluble without resort to methods that create new problems. We can do something to change ourselves so that we are not misunderstood and not inclined to misunderstand others, even if we cannot change the nature of the other fellow.

The individual who achieves the realization that he must understand others and, in turn, be understood is the one upon whom it devolves to keep a group of individuals working as a single team, and not as a group of individuals. We see practical application of this every day. Upon these people falls the responsibility of formulating the standards of the relationship of man to man in the organization. Man is easy to lead but hard to drive. He will follow with enthusiasm the one who provides him with an objective or a sense of mission, but he will not be driven by being lashed from the rear.

Kindness and consideration have, many times, saved the pride and respect of an individual when frankness would have destroyed his ego and made him resentful. One of the best loved editors in the United States, Grove Patterson of Toledo, Ohio, was remembered by every young journalist because of the care with which he supported every young man's pride. A young reporter would come to him, full of enthusiasm, with an idea. Although Mr. Patterson would immediately recognize that the idea was not practical, he would say, "That's a corking good idea. Take it, work it out carefully, going over every aspect of it and bring it back to me." The cub reporter would begin examining his "stupendous" idea and as doubts began creeping into his mind, would begin hoping that Mr. Patterson would forget about the subject. He usually did and thereby won the lasting affection of the young man.

Harmony and intelligent teamwork are greatly increased when a person or group of persons is given a real and compelling motive for working with others. Once upon a time, a Second Lieutenant reported for duty and after the Colonel had talked to him in a friendly manner, said, "Well, Colonel, we are going to get along fine." The Colonel's reply was, "Yes, young man, and you are going to do all the getting." His dignity had been offended and he missed an opportunity to exercise proper guidance. Motivation or a sense of purpose must be tactfully presented and in such a way that the individual feels he is part of a team doing something constructive. People do not achieve great solidarity or persevere at it simply because they are together. The important thing is that each member of the group, to a certain extent, incorporate into his life the thoughts, desires and interests of the others. We know that God has never created two people alike. Each individual wants to be treated with dignity and respect. He desires recognition. He wants the approval of his fellow workers and to be accepted by them. One of the best known Commanders in the Pacific War made it a point that when any member of his command, however unimportant, was promoted, decorated or given any recognition, he would write a letter to the man's wife or parents and tell them how proud he felt. Because of the many small things he did, he commanded the love and devotion of all his men.

Of Major General Henry T. Allen, who commanded our First Army of Occupation in Germany, a distinguished contemporary once said, "It surprised us that Allen did so well; in the

old Army he was regarded as a swashbuckler." Maybe that was because he was an old cavalry man and not only liked to strut but insisted that his men "stand tall" right down to the last file. He was infinitely considerate of the dignity of each man and disciplined himself to further their growth and show them some mark of his thoughtful regard. His rich, understanding humanity warmed his men and each was the better for having known him.

A preventive measure to insure good human relations is to be tolerant of the opinions of others. We recognize that other people are just as competent on their jobs as we are. They must be given the opportunity to develop their potentialities and to grow, and to utilize what they have to offer. They must be given the opportunity to help in the solving of problems in the organization. To many people, the opportunity to assist in arriving at a solution is a challenge that gives them the feeling of importance. Excessive supervision creates doubts, fears, shyness and discontent in people. To have someone breathing down your neck at all times is not a satisfying thing. Where people have been given the opportunity to show what they can do, they have done a far better job and have been much more effective. A relation of mutual confidence and loyalty must be permitted to develop. An employee must have faith in his superior.

I believe that the keystone of good human relations—person to person and group to group—is tolerance or recognition of rights of others regardless of how distasteful they may be to us.

The best way to gain a clear insight into any question in which there is a difference of opinion is to discuss it amicably. Nothing fosters friendship faster than getting a man to discuss his family, golf game, or his job. Many an executive has won the loyalty and devotion of his employee simply because he has taken the time to ask about a daughter away at school or to extend congratulations on the arrival of an heir.

I do not know if growing old makes us more cynical, more exacting, more critical or just more observing. It is my observation that certain basic qualities which have always been held high in our estimation need to be commented upon because of their intrinsic value in making human beings better individuals, better members of any organization and better citizens of this country. We have seen a segment of our people living on the negative side of life—believing that they are entitled to everything they desire without exerting any effort on their part. A whole generation has been raised in the belief that they should be given what they want, say what they please and do whatever his or her poor, undeveloped mind dictates. What has happened to loyalty, responsibility, integrity, courage and discipline?

There is within all men a strong desire to do the things they do in their own way and to express their desires in words. There is no quarrel with this. It is part of our way of life guaranteed to every American by the basic law of the land. When criticism becomes loose, thoughtless and malicious, then we have a corresponding, disruptive effect on the group. A constant questioning of individual judgement leads to loss of efficiency, instability and chaos.

The life of any socially upright individual is organized around a few basic loyalties: loyalty to self, loyalty to beliefs, loyalty to country, loyalty to friends and loyalty to humanity in general. The amount of satisfaction that an individual gets out of life is in direct proportion to his faithful adherence and service to these loyalties. Most important, he must keep faith with himself. Failing in this, he cannot be loyal to anyone or anything.

We are concerned with loyalty in our daily lives as we go about our day-to-day tasks and our relationships with our superiors and subordinates. It is a fundamental element of self-discipline; a willing compliance with the plans of the superior, the rules and regulations that govern the organization for which we work and an unfailing devotion to a cause.

It is not a blind and servile service to the letter of the rules and regulations but rather an active, intelligent and willing effort to carry out the intent of the superior to the best of our ability. Once a decision is made, it receives the complete and energetic support of the subordinate. The individual that can be depended upon to carry out only those decisions of which he approves is worthless to an organization. In any group, there are those who, because of heredity and environment, have such a creed of indomitable integrity that there is no doubt as to their performance of duty. Of this type, we have countless examples in public life and in our extensive corporate structures.

But, what about the plain, simple, everyday John Doe who is not blessed with this lofty, spiritual heritage; the person who was born on "the wrong side of the tracks"; the individual who is a product of the rough, tough, everyday world with all its vicissitudes and misfortunes? Are we to ignore him or concede with the melancholy poet that "God looks for no lustre from the minor stars"?

Obviously, there cannot be any doubt that the standards of loyalty and devotion expected,

must be the highest from one and all under all circumstances. It is just as obvious that these people must be treated with patience and understanding. We must recognize that their standard of performance will be conditioned by the conduct of their superiors. The superior who sets an example of loose criticism, especially in the presence of those people who are directly under his supervision cannot be surprised if he gets from those people only a similar lack of loyalty. At the same time, it is somewhat of an American tradition "to blow off steam" and griping does not necessarily indicate lack of loyalty. Although the griping may be the result of an immediate frustration, or it may be nothing more than an impatient expression, constant griping is indicative of disaffection. It is well to bear in mind the admonition of Elbert Hubbard:

"If you work for a man, in Heaven's name, work for him; speak well of him and stand by the institution which he represents. Remember —an ounce of loyalty is worth a pound of cleverness. If you must growl, condemn and eternally find fault, why—resign your position and when you are outside, damn to your heart's content—but so long as you are a part of the institution, do not condemn it; if you do, the first high wind that comes along will blow you away, and probably you will never know why."

Loyalty works both ways: loyalty to one's organization and to the people who comprise that organization. Each individual expects to be treated as a responsible adult and not as a schoolboy. He has rights and expects that these rights will be respected and safeguarded by his superior, the organization and his fellow-workers. He is ambitious and this ambition must be given every reasonable opportunity to express itself and receive recognition.

He has imagination and expects that imagination to be stirred and fostered by solicitation of his advice and an attentive ear to his suggestions. He has a sense of fair play and it must be honored. When he has a grievance he must feel that it will be listened to and the redress will be just. He has personal dignity and it must not be affronted, mocked or ridiculed. He has pride and it must be satisfied by making him feel that he is a respected part of a superior organization.

An individual becomes loyal because loyalty has been given him. He learns to serve an ideal because an ideal has served him.

Colonel S. L. A. Marshall sums it all up in his book, *Men Against Fire*:

"Once again, however, it might be well to speak of the importance of enthusiasm, kindness, courtesy, and justice, which are the safeguards of honor and the tokens of mutual respect between man and man. This last, there must be, if men are to go forward together, prosper in one another's company, find strength in the bonds of mutual service, and experience a common felicity in the relationship between the leader and the led. Loyalty is the big thing, the greatest battle asset of all. But no man ever wins the loyalty of troops by preaching loyalty. It is given to him as he has proved the possession of the other virtues. The doctrine of blind loyalty to leadership is a selfish and futile dogma except as it is ennobled by higher loyalty in all ranks to truth and decency.

"War is a much too brutal business to have room for brutal leading; in the end, its only effect can be to corrode the character of men, and when character is lost, all is lost. The bully and the sadist serve only to further encumber an army; their subordinates must waste precious time clearing away the wreckage that they make.

"The good company has no place for the officer who would rather be right than be loved, for the time will quickly come when he walks alone, and, in battle, no man can succeed in solitude."

What about the future? History has taught us that great industrial and economic changes were always followed by major adjustments in man's relationship to man. The atomic age has opened vast new frontiers and horizons. This, of necessity, must be accompanied by social change the exact form of which cannot be predicted at present. But one thing is sure and that is that the basic values of loyalty, responsibility, integrity, courage and discipline will remain with us as long as there is organized society and that it is these values which will shape the social concepts and the social structures of the future.

Toxicologic Studies on Organic Sulfur Compounds

1. Acute Toxicity of Some Aliphatic and Aromatic Thiols (Mercaptans)*

EDWARD J. FAIRCHILD, Ph.D. and HERBERT E. STOKINGER, Ph.D.

Occupational Health Program, Bureau of State Services, Public Health Service, U.S. Department of Health, Education, and Welfare, 1014 Broadway, Cincinnati, Ohio

TOXICOLOGIC studies of organic sulfur compounds have been initiated as the result of their increased usage in industry and continually increasing discovery of new compounds which emphasize the multiplicity of these materials as constituents of petroleum.[1, 2, 3, 4] The majority of sulfur present in crude oil is organic, the total quantity of which is relatively large in distillates from various high-sulfur petroleums. Past estimates for thiols alone place a value of from 150 to 200 tons lost per day from naphthas refined in this country.[5]

The presence of sulfur compounds other than sulfur dioxide and hydrogen sulfide in the atmosphere as contaminants in certain types of air pollution have, until very recently, received but little attention. Investigations in the Los Angeles area are the first attempts toward a comprehensive study of materials released to the atmosphere by petroleum refineries.[6] Eight per cent of the oil-refining capacity of the United States is located in this area. It has been assumed that refinery processes convert all sulfur compounds in petroleum to hydrogen sulfide and sulfur dioxide. No information is available on the exact nature of materials in refinery emissions. In addition to small amounts of particulate matter, NO_2, CO, and hydrocarbons, sulfur compounds occur, presumably consisting of predominantly low-boiling, stable thiols and sulfides, as well as H_2S and SO_2.[6, 33] Other sources of atmospheric contamination by sulfur compounds, possibly some organic, are paper mills, zinc plants, lead, copper and nickel smelters, and coal combustion. The annual losses of sulfur from products discharged to the atmosphere from these industries are of staggering proportions.[7] Similarly, fuel oil and gas burning, motor exhaust fumes and photochemical synthesis cannot be overlooked as possible sources of organic sulfur compounds.

A search of the literature revealed a paucity of information on thiol toxicity and that which is reported is often conflicting. Bonsib,[8] in a summary review of the use and toxicity of methyl, ethyl and butyl mercaptans, and the precautionary measures to be observed, drew attention to the conflicting information concerning these thiols. Oswald[9] states that mercaptans and sulfides properly belong in a class with hydrogen sulfide since they are so closely related chemically and have a paralyzing action similar to that of hydrogen sulfide. The effects of methanethiol upon mice, guinea pigs and rabbits were studied by DeRekowski[10] who reported it to be "very toxic" regardless of the method of administration. He found it to be considerably less toxic than hydrogen sulfide, however. Frankel[11] reported similar toxicity for methanethiol, whereas others[12] found its toxicity to be of the same magnitude as that of hydrogen sulfide. In a comparative study[13] of hydrogen sulfide and methyl mercaptan toxicity, the 10–20 minute fatal concentrations for rats were found to be 0.1% by volume (1.5 mg/l) and 1% by volume (20 mg/l) respectively. All investigators arrived at a mutual conclusion, however, in that methanethiol acted upon the respiratory center and produced death by respiratory paralysis. According to Flury and Zernik[14] ethanethiol in a concentration of 3000 ppm is not injurious to dogs, but a level of 10,000 ppm produced convulsions and paralysis of the respiratory center. Sayers, et al.[15] compared the effects of ethanethiol inhalation with those of hydrogen sulfide, and by analogy with the butyl homologue they conclude that more than 733 ppm of ethanethiol is required to kill dogs in 30 minutes. A report by Katz and Allison[16] states that butanethiol vapors are harmless, whereas more recent work[17] found the lethal concentration for dogs exposed 30

* Presented before the 18th Annual Meeting of the American Industrial Hygiene Association, April 25, 1957, St. Louis, Missouri.

minutes to be 2.7 mg/l (733 ppm). This is equivalent to approximately 0.1% by volume and is in agreement with the concentration of hydrogen sulfide required for a similar response.[8] The Army Chemical Center conducted studies[18] with a thiol mixture (propyl 24%, butyl 55% and amyl 21%) and found it slightly toxic to rats, rabbits and dogs, but concluded that inhalation presents a moderately severe hazard (LD_{50} for rats exposed four hours was 4100 ppm) because of the high volatility of the material and its production of tracheitis and pneumonitis.

Information concerning chronic toxicity of thiols is almost nonexistent. A single study[34] is cited for rats exposed to butanethiol vapors (40–50 ppm), six hours per day, five days per week for six months in which time three of six animals died. This study was apparently the basis for a threshold limit of 10 ppm for butanethiol.

Data are likewise meager regarding thiol toxicity in man. The U. S. Bureau of Mines conducted an investigation wherein 11 pounds of ethyl mercaptan per million cubic feet of air was introduced into the air of rooms occupied by sleeping persons; no ill-effects were noted after exposure of one-half to several hours.[17] Cristescu,[19] however, cites cases in which it was found that the inhalation of thiols produced nausea, vomiting, and diarrhea, as well as temporary excretion of albumin, casts and blood in the urine. In the same report Cristescu cites a case of acute thiol poisoning. A worker was seriously affected by inhaling unknown quantities and kinds of thiols while working in an oil refinery in Ploesti, Roumania. The worker, after an estimated two-hour exposure, was found unconscious and deeply cyanotic. Subsequent to treatment, observation and release, the patient was readmitted two weeks following exposure complaining of chest pains and violent coughing. Reexamination showed a lung abscess which was, in the words of the author "... nothing but a simple coincidence, probably favoured by the bronchitic state and a hypothetic anergisant effect of this poisoning."

Experimental Procedure

MATERIALS TESTED:

(a) Ethanethiol (ethyl mercaptan):
$CH_3CH_2 \cdot SH$,
Molecular weight: 62.13, Density (d 20/4): 0.8391,
Boiling point: 34.7°C.

(b) 1-Propanethiol (n-propyl mercaptan):
$CH_3(CH_2)_2SH$,
Molecular weight: 76.15, Density (d 20/4): 0.8337,
Boiling point: 67.5°C.

(c) 2-Methyl-1-propanethiol (isobutyl mercaptan): $(CH_3)_2CHCH_2SH$,
Molecular weight: 90.18, Density (d 20/4): 0.8357,
Boiling point: 88.7°C.

(d) 2-Methyl-2-propanethiol (tertiary butyl mercaptan): $(CH_3)_3CSH$,
Molecular weight: 90.18, Density (d 20/4): 0.8357,
Boiling point: 64.2°C.

(e) 1-Butanethiol (n-butyl mercaptan):
$CH_3 \cdot (CH_2)_3SH$,
Molecular weight: 90.18, Density (d 20/4): 0.8333,
Boiling point: 98.4°C.

(f) 1-Hexanethiol (n-hexyl mercaptan):
$CH_3 \cdot (CH_2)_5SH$,
Molecular weight: 118.23, Density (d 20/4): 0.8490,
Boiling point: 149–50°C.

(g) Methyl heptanethiol (tertiary octyl mercaptan): $C_8H_{17}SH$,
Molecular weight: 146.28, Density (d 60/60°F): 0.8513,
Boiling point: 140.5°C. (Position of $—CH_3$ and —SH on 7-carbon chain is unknown).

(h) Benzenethiol (phenyl mercaptan):
$C_6H_5 \cdot SH$,
Molecular weight: 110.17, Density (d 20/4): 1.078,
Boiling point: 169.5°C.

(i) α-Toluenethiol (benzyl mercaptan):
$C_6H_5 \cdot CH_2SH$,
Molecular weight: 124.19, Density (d 20/4): 1.058,
Boiling point: 194–5°C.

Each of these compounds is a colorless liquid, with the exception of methyl heptanethiol, which is a pale straw color. All are slightly acidic because of the —SH group. Their solubilities vary but little, the lower members, up to C_5, being slightly soluble in water, whereas the higher homologues are insoluble. All are soluble in alcohol and acetone, soluble to slightly soluble in olive and peanut oil, and slightly soluble to insoluble in propylene glycol. The thiols have characteristic disagreeable odors; those of alpha-toluenethiol and benzenethiol are slightly nauseating. Gas chromatographic analysis showed six of the thiols to be essentially pure compounds; the three thiols with the highest boiling points could not be analyzed in this manner. The benzenethiol was highest purity Eastman Grade. Alpha-toluenethiol and methyl heptanethiol had calculated purities of 97 and 96 per cent, respectively. According to the manufacturer, the latter probably contains small amounts of compounds of a carbon chain below and above the molecular weight normally identified with

the name. Samples of each of the compounds were subjected to infrared analysis and spectrograms have been retained for future reference.

METHODS

All experimental animals used in these studies were obtained from a local commercial breeder and maintained in our housing facilities at least one week prior to use. All experiments were conducted with males, and, excluding actual preparation and exposure time, water and a basic diet consisting of Rockland Rat Diet (for rats and mice) and Rockland Rabbit Ration were always made available. Routine sacrifice of rodents was accomplished by separation of the cervical vertebrae. Rabbits were sacrificed by overdoses of Nembutal injected intravenously. Other than those previously designated for early sacrifice, all animals were kept at least two weeks following an experiment. Most animals were held for observation for a month prior to being either destroyed or sacrificed for study. Tissue specimens were submitted for pathologic examination after having first been examined grossly and preserved in either buffered formalin or Zenker's fixative. LC_{50} values by inhalation were calculated by the method of Miller and Tainter[20] using logarithmic-probit graph paper, whereas the LD_{50} for all other routes of administration was calculated by the method of Weil.[21]

Intraperitoneal Injection. Groups of at least five (occasionally 10) Wistar-derived rats, each weighing on the average 200 ∓20 grams, were injected intraperitoneally at dosage levels differing by a factor of either 1.26 or 2.0 in a geometric series, according to Weil. In all cases at least four groups were used to obtain data for calculation of the LD_{50} by single injection. Because of the small dosages required for some of the more toxic thiols, these were administered as solutions in various vehicles. Ethanethiol was administered both undiluted and as a 10 and 50% v/v solution in olive oil; single injections of benzenethiol and methyl hepthanethiol were given as 5% v/v solutions in ethanol, and repeated subacute doses as 2% v/v solutions were administered three times weekly for three and four weeks, respectively.

Oral Administration. Rats were used of the same stock and weight as those previously described. Each of the thiols was administered by gavage to at least four groups of five rats each. Seven of the compounds were given undiluted, whereas benzenethiol and methyl heptanethiol were administered in ethanol as an 8% v/v solution. Rats were dosed at levels in geometric progression (factor 1.26 or 2.0) by introducing measured amounts of the materials or solutions from a hypodermic syringe and blunted needle (8 cm, 18 gauge) which was passed through the esophagus into the stomach.

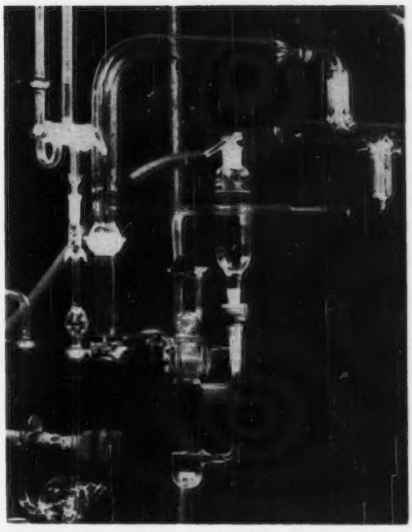

FIGURE 1. Apparatus for Generation of Thiol Vapors.

Inhalation Exposure. Five rats and ten mice (Swiss-derived, usually averaging 25 to 28 grams) comprised the groups of experimental animals that were subjected to the inhalation of thiol vapors. Four-hour exposure periods were the standard operating procedure, except when obvious departures from this procedure were indicated. Hamsters were used in some comparative tests.

The generation of thiol vapors was accomplished by either of two methods: (1) bubbling a stream of nitrogen gas (to prevent possible oxidation to sulfide) through a midget fritted-glass bubbler, which contained the liquid thiol, or (2) by passage of nitrogen into a borosilicate glass nebulizer which contained the thiol. These are shown in Figure 1 which indicates how each was assembled as part of the exposure apparatus. The bubbler (approximate 60 ml, with a 24/40 ground joint and a coarse porosity disc) was modified by adding an upright side-arm delivery tube (8 mm O.D. × 6 in.) with a 10/30 ground joint distal end into which a separatory funnel (60 ml cylindrical with teflon-glass valve) was inserted. The side-arm was high enough to permit partial submersion of the bubbler if a constant temperature bath was required. The separatory funnel served as a reservoir for the thiol being vaporized, and the teflon valve permitted easy delivery of the contents into the bubbler.

The nebulizer was modified by addition of side-arm tubes to make it adaptable to requisites of the experiments. An upright tube (8 mm O.D. × 6 in.) with a distal socket joint (12/5) was annealed to, and confluent with the jet inside the nebulizer, thus permitting the introduction of nitrogen (extreme left—Fig. 1). A second tube identical with the first entered one side of the nebulizer approximately one centimeter from the bottom and served as an inlet for the thiol which was delivered dropwise from a burette (Koch, 10 ml). The removable burette tip protruded about 5 cm into, and was annealed with the expanded (2 cm O.D.) bulb-shaped end of a short length of glass tubing (8 mm O.D. × 3 cm) whose opposite end had a ball joint (12/5). The tip, therefore, connected the burette with the side-arm delivery tube of the nebulizer, thus permitting a flow of liquid from the burette into the nebulizer. The flow was regulated by observing the drop rate from the bulb-encased tip. A third arm (2 cm O.D.), through which the vapor passed, was annealed to the front of the nebulizer and projected abruptly upward so that its distal end, a 28/15 socket, was the same height as the socket-ends of the smaller side-arm tubes. These alterations permitted a desired feed rate of liquid from the reservoir of the burette and this in turn permitted the maintenance of a constant volume of liquid in the nebulizer. This arrangement facilitated the calculation of the total amount of liquid delivered and vaporized in a unit of time.

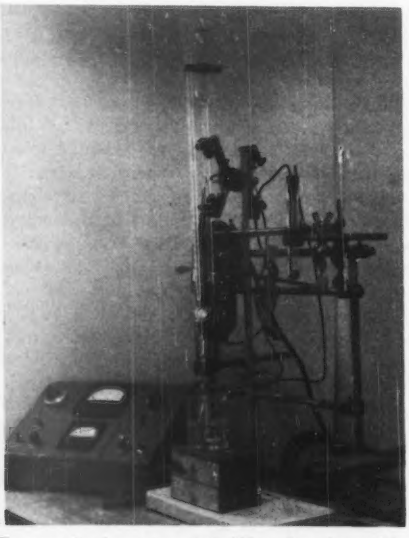

FIGURE 2. Amperometric Titration Assembly

It also permitted complete submersion of the vaporizing unit in a constant temperature bath. Each of the described methods was used interchangeably, but with some of the lower boiling-point thiols the bubbler proved more manageable and gave more uniform chamber concentrations than the nebulizer.

Desired exposure concentrations of thiols were maintained in a glass chamber[22, 23] of approximately 18-liter capacity by varying the ratio of volume flow (liters/minute) of compressed air and compressed nitrogen. Prior to entering the chamber, the compressed air was scrubbed by passage through a fritted bubbler containing potassium dichromate in concentrated sulfuric acid, thence through a column of glass wool which was followed by a column of Drierite. From this it went into a mixing tube which received the thiol vapors by another inlet; the mixture then passed into the exposure chamber.

Sampling and Analysis of Exposure Atmosphere. During exposure periods the concentrations of thiols within the chamber were determined routinely by absorption of vapors in either isopropyl alcohol or acetone containing an excess of silver nitrate and titrating the uncombined silver amperometrically according to the method of Grimes, et al.[24] The electrometric analysis assembly (Figure 2) consisted of a Sargent Ampot which served as the current-measuring circuit, a bulb-type rotating platinum electrode, a sleeve-type calomel pencil electrode (the reference electrode), a 2 ml Koch microburette, and a friction cone-drive stirrer which was modified for use as an electrode holder. The modification consisted of an arrangement whereby the electrical circuit between the rotating platinum electrode and the current measuring Ampot was completed by passage of current through the shaft of the stirrer.

Samples for analysis were collected in an Erlenmeyer flask (125 ml capacity with a 24/40 glass stopper which had 12/5 joints on the inlet and outlet) containing an excess of silver nitrate of known normality (approximately 0.01 N) in 50 ml of acetone or isopropyl alcohol; the choice of solvent was determined by the thiol used and its solubility therein. After the thiol vapors had been metered through this solution, the resulting precipitate (silver mercaptide) and other contents were quantitatively washed from the flask and the excess silver titrated amperometrically with standard dodecyl mercaptan (approximately 0.044 N). Prior to use, the mercaptan was purified by the method of Grimes.[24]

Accuracy of the sampling technique and analytical procedure as applied to this work

was tested by vaporizing known amounts of thiols using the nebulizer-burette arrangement and found to be within 2% of that calculated. In tests without animals in the chamber, thiol concentrations at the outlet were 1 to 2% lower than those sampled at the inlet. This was determined for chamber-air change rates of one per minute (20 1/min) and one per two minutes (10 1/min). With animals, concentrations at the outlet were 3.7 and 8.2% lower than at the inlet using the same respective air-change rates. Chamber concentrations during tests were relatively uniform after the first 30 minutes. Variation between extremes of vapor concentrations measured during any one test was never greater than 15%, usually being about 9%. The minimum variation between concentrations (high and low) for any one test was 0.4%, while the mean variation for all exposures was about 4%.

Ocular Exposure. In general, techniques of tests followed those of Draize and Kelly[25] for testing the irritation of substances on the eye mucosa of rabbits. The initial exposures employed one rabbit (New Zealand strain) for each compound, subsequent tests being governed by results of the first. One-tenth ml of thiol was instilled in the conjunctival sac of the right eye and the left served as a control. If initial irritation reactions were slight to moderate but persisted after 24 hours, the thiol was administered to the control eye which in turn was washed copiously with water approximately five seconds after instillation. In cases of severe irritation additional rabbits were used to determine the effects of water and/or solutions of silver nitrate as an eyewash. Ocular reactions were read with a hand slit lamp at 24, 48 and 72 hours, usually at four days and always at seven days after treatment. If residual injury persisted beyond the first week, readings were made at intervals until the condition either cleared or became static.

Percutaneous Application. Cutaneous LD_{50} values for rats, five groups of five each, were calculated from mortality data obtained by single application of undiluted compounds (dosage levels in geometric progression) to a clipped area of the animals' backs. In like manner, approximate LD_{50} values were determined for rabbits in three groups of two each. In this method areas of approximately 3 cm^2 and 6 × 10 cm of the upper midbacks of rats and rabbits respectively were clipped as close to the skin as possible, care being taken to avoid abrasions and cuts. Animals were then placed in individual retainers and measured amounts of materials were delivered dropwise upon the clipped areas of the skin, exercising care in restricting the entire dosage to these areas.

Results

Intraperitoneal Injection. As seen in Table I, benzenethiol and methyl heptanethiol were more toxic by a considerably larger factor than the other seven thiols when tested in rats. They were approximately 20 times more toxic than the next most toxic, ethanethiol, and approximately 90 times more toxic than the least toxic, 2-methyl-1-propanethiol. On the basis of a single, acute, intraperitoneal dose, therefore, benzene- and methyl heptanethiol might be considered as "highly" toxic, whereas the 7 other thiols range from "moderately" to "slightly" toxic. Delayed toxicity with intermittent mortality was observed to some extent for all but alpha-toluene- and methyl heptanethiol. This is seen after comparison of LD_{50} values for the 24- and 48-hour and 15-day periods; the 24-hour LD_{50} values for compounds other than alpha-toluene- and methyl heptanethiol are approximately 2 to 1.5 times greater than are the respective 15-day values. As indicated by the cumulative mortality data, much of the delayed toxic response occurred subsequent to the 48-hour post-injection period.

The course of thiol toxicity, at least for ethanethiol, was considerably altered when administered in an oil vehicle. Injections of 840 mg/kg as a 50% v/v solution in olive oil did not produce 100% mortality (10 rats) until 64 hours post-injection and the first death did not occur until 42 hours. Undiluted ethanethiol in this dosage produced 100% mortality in 3 to 7 hours (Table I). These differences are apparently a function of absorption rates. It was found, however, that this dosage of thiol-oil mixture did not produce any mortality if administered approximately 24 hours after preparation of the solution. This suggested a reaction between the thiol and the olive oil, possibly between the —SH group and the double bonds of the unsaturated components of olive oil. In an effort to confirm this, ethanethiol was given as 50% v/v solutions in undecylenic (unsaturated) and undecylic (saturated) acids. The toxicity of ethanethiol (840 mg/kg) in these vehicles, as judged by mortality per unit time, was not appreciably altered from that found for the undiluted thiol. Thus no light was thrown on the possible action of the thiol in olive oil. Similarly, ethanethiol (same dosage) was given as 50% v/v solutions in propylene glycol and petrolatum and produced 100% mortality (5 rats) in 20 to 50 and 30 to 40 hours, respectively.

TABLE I

Toxicity Data for Rats Following Single Intraperitoneal Injections of Thiols

Dose mg/kg	Cumulative Mortality Following Administration for Day					
	1	2	3	5	10	15
Ethanethiol						
105	0/10	0/10	0/10	0/10	0/10	0/10
210	0/10	0/10	2/10	3/10	4/10	4/10
420	4/10	5/10	7/10	9/10	10/10	
840	10/10 (All dead in 3 to 7 hrs.)					
1680	5/5 (All dead in 2 hrs.)					
LD_{50} (mg/kg)	450	420				226
Confidence Limits	359–564	331–532				180–283
Propanethiol						
209	0/5	0/5	0/5	0/5	0/5	0/5
418	0/5	0/5	1/5	1/5	1/5	1/5
836	1/5	3/5	4/5	5/5		
1672	5/5 (All dead 3.5 to 9 hrs.)					
LD_{50} (mg/kg)	1028	780				515
Confidence Limits	781–1298	556–1096				390–679
2-Methyl-1-propanethiol						
209	0/5	0/5	0/5	0/5	0/5	0/5
418	0/5	0/5	1/5	1/5	1/5	1/5
836	0/10	0/10	2/10	2/10	2/10	2/10
1672	5/5 (2/5 dead 6 hrs.; 5/5 dead 12 hrs.)					
LD_{50} (mg/kg)	1183					917
Confidence Limits	939–1487					575–1345
2-Methyl-2-propanethiol						
209	0/5	0/5	0/5	0/5	0/5	0/5
418	0/5	0/5	0/5	0/5	1/5	1/5
836	0/5	1/5	2/5	3/5	4/5	4/5
1672	5/5 (2/5 dead 2 hrs.; 5/5 dead 7 hrs.)					
3344	5/5 (5/5 dead 1/2 hr.)					
LD_{50} (mg/kg)	1182	1029				590
Confidence Limits	939–1487	780–1358				399–875
Butanethiol						
209	0/5	0/5	0/5	0/5	1/5	1/5
418	0/5	0/5	1/5	1/5	2/5	2/5
836	4/5	4/5	4/5	5/5		
1672	5/5 (All dead 5 to 8 hrs.)					
LD_{50} (mg/kg)	679					390
Confidence Limits	515–896					257–619
Hexanethiol						
212	0/5	0/5	0/5	0/5	0/5	0/5
424	1/5	1/5	1/5	1/5	2/5	3/5
848	4/5	4/5	5/5			
1696	5/5					
LD_{50} (mg/kg)	600					396
Confidence Limits	405–887					282–556

TABLE I. (*Continued*)

Dose mg/kg	Cumulative Morality Following Administration for Day					
	1	2	3	5	10	15
Methyl Heptanethiol[a]						
4.3	0/5	0/5	0/5	0/5	0/5	0/5
8.6	0/5	0/5	0/5	0/5	0/5	0/5
10.8	1/5	1/5	1/5	1/5	1/5	1/5
13.6	3/5	3/5	3/5	3/5	3/5	3/5
17.1	5/5 (3/5 dead 1/2 hrs.; 5/5 dead 7 hrs.)					
34.2	5/5 (3/5 dead 40 mins.; 5/5 dead 4 hrs.)					
LD_{50} (mg/kg)	12/9					
Confidence Limits	11.2–14.9					
Benzenethiol[a]						
6.7	0/5	0/5	0/5	0/5	0/5	0/5
13.5	0/5	0/5	0/5	0/5	2/5	2/5
27.0	3/5	3/5	3/5	5/5 (one dead 6 hrs. past 5 days)		
54.0	5/5 (All dead 1 hr. 10 mins.)					
108.0	5/5 (All dead 1 hr. 5 mins.)					
LD_{50} (mg/kg)	25.2					9.8
Confidence Limits	17.9–35.4					7.0–13.7
Alpha-Toluenethiol						
132	0/5	0/5	0/5	0/5	0/5	0/5
264	0/5	0/5	0/5	0/5	1/5	1/5
529	4/5	4/5	4/5	4/5	4/5	4/5
1058	5/5 (All dead 7 hrs.)					
LD_{50} (mg/kg)	429					373
Confidence Limits	325–566					252–553

[a] Administered as 5% v/v solution in ethanol.

If the solutions were administered 24 hours after preparation, no mortality resulted. Mechanisms involved in the interaction of these materials are yet unknown.

Thiols were found to have a common toxicologic property of being soporific, the degree ranging from mild stupor to heavy sedation. Relatively deep sedation was produced by maximal sublethal intraperitoneal doses of butane-, propane- and especially ethanethiol. The sedative action of these thiols persisted for one to several hours followed by a lethargic condition for varying periods of time. These conditions were slight for the aromatic thiols. The response of the thiol-injected rats was fairly uniform in that symptomatology of acute poisoning developed in the order of: restlessness, increased respiration, incoordination, muscular weakness, skeletal muscle paralysis in most cases (starting with hind limbs), heavy to mild cyanosis, lethargy and/or sedation, respiratory depression followed by coma and death in cases of lethal doses.

Methyl heptanethiol was an exception since it was shown to be a potent central nervous system (CNS) stimulant rather than a depressant. The material was fast-acting and produced symptoms 2 to 3 minutes post-administration. The initial observable response, regardless of dosage, was respiratory stimulation. For toxic doses this was followed within 3 to 5 minutes by a definite pattern of CNS stimulation, initially characterized by a "threshold effect"[26] consisting of localized minimal convulsive movements in the form of repeated facial and ear twitches. An increase in dosage (up to 5–8 mg/kg) invariably increased the intensity of this effect to produce single or repeated jerking movements resulting in pro- and/or retropulsive thrusts of the trunk. Usually after one or a series of these movements rats developed circumscribed clonic convulsions limited to forebody and forelegs, frequently resulting in assumption of a sitting "squirrel-like" position while "pawing the air." This response always occurred in the more toxic dose range (above 15 mg/kg) and developed into generalized clonic seizures involving both fore and hind limbs simultaneously usually causing a loss of the upright position. In cases of maximal nonlethal and lethal doses,

TABLE II

Toxicity Data for Rats Following Single Oral Dose of Thiols

Dose mg/kg	Cumulative Mortality Following Administration for Day					
	1	2	3	5	10	15
Ethanethiol						
210	0/5	0/5	0/5	0/5	0/5	0/5
420	0/5	0/5	0/5	0/5	0/5	0/5
840	2/5	2/5	2/5	2/5	3/5 (7th)	4/5 (11th)
1680	4/5	4/5	4/5	5/5		
3360	5/5 (All dead 4 to 7 hrs.)					
LD_{50} (mg/kg)	1034					682
Confidence Limits	667–1603					517–900
Propanethiol						
1327	0/5	0/5	0/5	0/5	0/5	0/5
1672	0/5	0/5	0/5	1/5 (4th)	1/5	1/5
2107	2/5	3/5	3/5	4/5 (9th)	5/5	
2654	3/5	5/5 (4/5 dead 42 hrs.; 5/5 dead 50 hrs.)				
3344	5/5 (1/5 dead 4 hrs.; 5/5 dead 10–20 hrs.)					
LD_{50} (mg/kg)	2362	2055				1790
Confidence Limits	2014–2770	1836–2300				1632–1963
2-Methyl-1-propanethiol						
1672	0/5	0/5	0/5	0/5	0/5	0/5
3344	0/5	0/5	0/5	0/5	0/5	0/5
6688	1/5	3/5	3/5	3/5	3/5	3/5
13376	3/5	4/5	4/5	4/5	4/5	4/5
26752	5/5 (3/5 dead 6 hrs.; 5/5 dead 10–20 hrs.)					
LD_{50} (mg/kg)	10,865	7168				
Confidence Limits	7009–16,850	4624–11,120				
2-Methyl-2-propanethiol						
1672	0/5	0/5	0/5	0/5	0/5	0/5
3344	0/5	0/5	0/5	0/5	0/5	0/5
6688	4/5	5/5 (4/5 dead 12 hrs.; 5/5 dead 32 hrs.)				
13376	5/5 (4/5 dead 7 hrs.; 5/5 dead 20 hrs.)					
LD_{50} (mg/kg)	5432	4729				
Confidence Limits	4117–7168	3756–5954				
Butanethiol						
1093	0/5	0/5	0/5	0/5	0/5	0/5
1327	0/5	2/5	2/5	2/5	2/5	2/5[a]
1672	1/5	2/5	3/5	3/5	4/5 (7th)	4/5
2107	1/5	4/5	4/5	4/5	4/5	4/5
2655	2/5	5/5 (5/5 dead 28–31 hrs.)				
3344	5/5 (All dead 6–20 hrs.)					
LD_{50} (mg/kg)	2575	1683				1500
Confidence Limits	2145–3090	1346–2105				1244–1809
Hexanethiol						
848	0/5	0/5	0/5	0/5	0/5	0/5
1068	0/5	0/5	0/5	1/5	1/5	1/5
1345	0/5	2/5	3/5	3/5	3/5	3/5
1696	0/5	2/5	4/5	5/5		
2137	3/5	5/5				
LD_{50} (mg/kg)		1580				1254
Confidence Limits		1347–1855				1084–1451

TABLE II. (*Continued*)

Dose mg/kg	Cumulative Mortality Following Administration for Day					
	1	2	3	5	10	15
Methyl Heptanethiol[b]						
34.2	0/5	0/5	0/5	0/5	0/5	0/5[c]
54.2	1/5	1/5	1/5	1/5	1/5	1/5
68.3	2/5	2/5	2/5	2/5	2/5	2/5
86.1	1/5	1/5	1/5	1/5	1/5	1/5
108.5	5/5 (All dead 3 hrs.)					
LD_{50} (mg/kg)	85.3					
Confidence Limits	69.6–100.2					
Benzenethiol[b]						
216	0/5	0/5	0/5	0/5	0/5	0/5
43.0	3/5	3/5	3/5	3/5	3/5	3/5
86.2	4/5	4/5	4/5	4/5	4/5	4/5
172.5	5/5 (All dead 5 hrs.)					
LD_{50} (mg/kg)	46.2					
Confidence Limits	29.8–71.6					
Alpha-Toluenethiol						
132	0/5	0/5	0/5	0/5	0/5	0/5
264	0/5	0/5	0/5	0/5	0/5	0/5
528	0/5	0/5	0/5	3/5	3/5	3/5
1056	3/5	5/5				
2112	5/5 (All dead 6–10 hrs.)					
LD_{50} (mg/kg)	985					493
Confidence Limits	702–1383					351–692

[a] One additional death on day 17.
[b] Administered as 8% v/v solution in ethanol.
[c] One death occurred day 19 from pneumonia.

2 to 3 clonic seizures occurred at 3 to 4–minute intervals. These were followed by one or more tonic seizures involving extensor tonus of both fore and hind legs. After this phase of seizure rats (generally) showed a marked degree of emprosthotonus. Exophthalmus with conjunctival congestion and slight to heavy salivation were associated with seizures. In cases of maximal lethal doses the animals often died in the emprosthotonic condition, but usually there was muscle relaxation, irregular labored breathing, coma and death.

Repeated Intraperitoneal Injections. The marked toxicity of benzene- and methyl heptanethiol indicated the desirability of determining the possible cumulative toxic effects of the material when injected repeatedly at low levels (1/5 LD_{50}). Accordingly, benzenethiol was administered to each of six rats in 9 doses of 3.5 mg/kg as a 2% v/v solution in ethanol over a three-week period. One rat died the seventh day. Similarly, methyl heptanethiol was administered to six rats in 14, 1/5 LD_{50} doses of 4.3 mg/kg (2% v/v solution in ethanol) during one month. One rat died on the seventh day (fourth injection) after several convulsive seizures. Neither this group of rats nor those injected with benzenethiol showed significant weight losses, although the averages were slightly lower than control rats. It was noted that all but one rat receiving methyl heptanethiol exhibited the typical threshold effects and light to moderate circumscribed clonic seizures. One thiol-injected rat displayed the toxic signs shortly after each of eleven of the 14 injections, two showed signs after each of nine injections and one rat responded with seizures after each of four injections. The intensity of response shown by any one rat varied during the injection schedule, but in no case did it appear to increase progressively with the number of injections.

Oral Administration. As seen in Table II, thiol toxicity was considerably less when administered by gavage than by the intraperitoneal route. Again, however, benzenethiol and methyl heptanethiol were markedly more toxic, having respective LD_{50} values approximately 10 and 6 times greater than the next most toxic substance, alpha-toluenethiol. Similarly, they were approximately 150 and 85 times, respectively,

TABLE III

Toxicity Data on Four Hour Inhalation Exposures of Rats and Mice to Thiol Vapors

Analyzed Conc'n ppm	Cumulative Mortality During and After Exposure							
	Rats				Mice			
	0–4 Hr.	24 Hr.	48 Hr.	15 Day	0–4 Hr.	24 Hr.	48 Hr.	15 Day
Ethanethiol								
2600	0/5	0/5	0/5	0/5	1/10	4/10	4/10	4/10
3150	0/5	0/5	0/5	0/5	4/10	7/10	7/10	7/10
3573	0/5	0/5	0/5	0/5	8/10	10/10		
4438	0/5	0/5	1/5	1/5	10/10			
4832	1/6	3/6	3/6	4/6	10/10			
4868	1/5	2/5	2/5	2/5				
5100	2/5	5/5						
5125	2/6	2/6	2/6	2/6				
LC_{50}		4870	4565	4420			2770	
Confidence Limits		4783–4957	4448–4682	4299–4541			2661–2879	
Propanethiol								
3050	0/6	0/6	0/6	0/6	0/20	0/20	4/20	4/20
4500	0/6	0/6	0/6	0/6	8/20	10/20	10/20	14/20
8340	3/6	4/6	4/6	4/6	20/20			
11260	5/6	6/6			10/10 (All dead 2 hrs. 15 min.)			
LC_{50} (estimated)			7300				4950	4010
2-Methyl-1-propanethiol								
10030	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
17600	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
21150	0/6	0/6	0/6	0/6	0/10	2/10	2/10	2/10
25065	0/6	0/6	0/6	0/6	0/10	1/10	1/10	2/10
LD_{50} (not calculable)								
2-Methyl-2-propanethiol								
11520	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
15465	0/6	0/6	0/6	0/6	0/10	3/10	4/10	4/10
25495	0/6	4/6	4/6	4/6	3/10	10/10		
LC_{50} (estimated)				22,200		16,750		16,500
Butanethiol								
2150	0/6	0/6	0/6	0/6	0/12	0/12	0/12	0/12
2450	0/5	0/5	0/5	0/5[a]	0/10	7/10	8/10	8/10
2860	0/5	0/5	0/5(13)	1/5	3/10	10/10		
3850	0/5	1/5	2/5	2/5[b]	5/10	8/10	10/10	
5460	1/5	4/5	4/5	4/5[c]	10/10			
6000	4/5	5/5			10/10			
LC_{50}		4460	4280	4020		2950		2500
Confidence Limits		4132–4786	3959–4601	3656–4384		2824–3076		2437–2563
1-Hexanethiol								
220					0/12	0/12	0/12	0/12
353					0/12	2/12	3/12	3/12
456	0/6	0/6	0/6	0/6	0/10	3/10	3/10	3/10
822	0/6	1/6	1/6	2/6	3/10	7/10	7/10	8/10[d]
1224	1/5	3/5	3/5	3/5[e]	4/10	9/10	10/10	
1260	0/6	2/6	4/6	4/6	6/10	10/10		
1475	4/5	4/5	5/5		10/10			
LC_{50}		1200	1145	1080		610	550	528
Confidence Limits		1115–1285	1044–1236	930–1230		548–672	487–613	470–586

TABLE III. (*Continued*)

Analyzed Conc'n ppm	Cumulative Mortality During and After Exposure							
	Rats				Mice			
	0–4 Hr.	24 Hr.	48 Hr.	15 Day	0–4 Hr.	24 Hr.	48 Hr.	15 Day
Methyl Heptanethiol								
38	0/5	0/5	0/5	0/5	0/10	0/10	0/10	0/10
40	1/6	1/6	1/6	1/6	2/10	2/10	2/10	2/10
44	1/5	1/5	1/5	1/5	4/10	4/10	4/10	4/10
55	3/5	3/5	3/5	3/5	9/10	9/10	9/10	9/10
64	5/5 (All dead 3 hrs. 10 min.)							
					10/10 (All dead 3 hrs.)			
78	6/6 (All dead 2 hrs. 50 min.)							
					10/10 (All dead 1 hr. 35 min.)			
110	6/6 (All dead 2 hrs. 45 min.)							
LC_{50}	51				47			
Confidence Limits	46.5–54.5				45.3–48.7			
Benzenethiol								
20	0/5	0/5	0/5	0/5[c]	0/10	0/10	0/10	0/10
31	0/10	0/10	0/10	5/10	0/10	0/10	3/10	7/10[f]
41	0/6	0/6	1/6	4/6	4/10	7/10	8/10	10/10[f]
52	0/5	0/5	2/5	5/5	9/10	10/10		
79	0/10	1/10	3/10	10/10[g]	5/5 (2 hrs. post exposure)			
132	10/10 (All dead 3 hrs.)							
LC_{50}			59	33		47	35.5	28
Confidence Limits			50.7–67.3	29.6–36.4		43.4–50.6	32.4–38.6	24.8–31.2
Alpha-Toluenethiol								
58	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
98	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
130	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
145	0/6	0/6	0/6	0/6	0/10	0/10	0/10	0/10
185	0/6	0/6	0/6	1/6[h]	3/10	3/10	3/10	6/10[j]
235	0/6	0/6	0/6	1/6[i]	1/10	10/10		
LC_{50}			Not calculable[k]			195 (est.)		178 (est.)

[a] 5th rat died day 17—pneumonia.
[b] one additional death day 19—pneumonia.
[c] one additional death day 17—pneumonia.
[d] 8th mouse died day 13—pneumonia.
[e] 3 died day 6, 4th died day 11, 5th died day 16—3 died from pneumonia.
[f] 2 deaths from pulmonary infections—some pneumonia.
[g] 7 deaths between days 3 and 8—some pneumonia.
[h] died day 13 with pneumonia.
[i] died day 7.
[j] one died day 3, two died day 4.
[k] higher concentrations not used because of heavy condensation and other technical difficulties.

more toxic than the least toxic 2-methyl-1-propanethiol. Classification[27] in order of decreasing toxicity for the oral route shows but one compound, benzenethiol, as "highly" toxic, methyl heptane- and alpha-toluenethiol as "moderately" toxic, ethane-, hexane-, butane-, propane-, and 2-methyl-2-propanethiol as "slightly" toxic, and 2-methyl-1-propanethiol as "practically nontoxic." Latent toxicity was not observed so frequently orally as intraperitoneally, but was observed to some extent for all except methyl heptanethiol and benzenethiol.

Signs of acute toxicity for the oral route were, with few exceptions, essentially the same as those described for the intraperitoneal route. The sedative action characteristic of most of the thiols was evident, but was especially pronounced for hexanethiol; in maximal sublethal doses rats were in deep comatous sleep for approximately 48 hours. In addition, diarrhea was pronounced for those compounds administered in relatively large doses.

Inhalation Exposure. Mortality data in Table III shows that mice were more susceptible to

the toxic action of thiols than were rats. Comparison of 15-day post-inhalation LC_{50} values indicated that thiol vapors, except those of benzene- and methyl heptanethiol, were approximately twice as toxic for mice. The range of toxicity (expressed as per cent of dose) was greater than that found for either the intraperitoneal and oral routes; benzene- and methyl heptanethiol were more toxic than least toxic 2-methyl-2-propanethiol by a factor of approximately 600 and 400, respectively. Toxicity classification[27] based on single 4-hour exposures shows benzene- and methyl heptanethiol as "highly" toxic, alpha-toluene- and hexanethiol as "moderately" toxic, butane-, ethane- and propanethiol as "slightly" toxic, and 2-methyl-2-propane- and 2-methyl-1-propanethiol as "practically nontoxic." A higher incidence of latent toxicity was observed for rats than for mice, as seen in Table III, but neither species showed this from exposures to methyl heptanethiol. An especially marked degree of latent mortality was observed in both rats and mice exposed to benzenethiol vapors.

Both rats and mice exhibited signs of intoxication which, with the exception noted below were similar to those observed in rats for other routes of administration. Maximal sublethal and lethal concentrations of thiols other than methyl heptanethiol induced characteristic symptoms of toxicity, i.e., increased respiration and restlessness (hyperactivity in mice), incoordinated movement and staggering gait, muscular weakness, partial skeletal muscle paralysis beginning in hind limbs, light to severe cyanosis, tolerance of prone position, and mild to heavy sedation. Fatal responses usually followed one of two patterns: (1) animals exposed to maximal lethal concentrations died from respiratory arrest while in or shortly after removal from the chamber, and (2) those animals exposed to minimal lethal concentrations died while in a semiconscious condition of long duration. Animals exposed to ethane-, butane- and hexanethiol very often remained in a semi-conscious condition of sedation and lethargy 4 to 6 hours postexposure before showing signs of recovery. Occasionally a period of deep lethargy of 18 to 28 hours intervened before visible signs of recovery occurred from exposure to butane- and hexanethiol. On the other hand, the aromatic thiols induced some lethargy and sedation which was quickly terminated upon exposure to normal atmosphere.

Inhalation of methyl heptanethiol vapors produced signs of intoxication similar in type but varied in developmental pattern from those described for the intraperitoneal and oral routes. The appearance of the initial threshold effects and subsequent seizures were dependent upon vapor concentrations to which the animals were exposed, whereas for other routes the response of CNS stimulation appeared 6 to 10 minutes post-administration irrespective of dosage. Exposure to 110 ppm of vapor induced several close interval (2–3 minutes apart) threshold effects and mild circumscribed clonic seizures within 10 to 15 minutes. Exposure to 64 to 78 ppm induced the initial responses in approximately 20 to 30 minutes, with an interval of several minutes between effects. Low concentrations required 45 minutes to 1.5 hours exposure prior to initiation of the first observable threshold effect, and occasionally no response occurred until the last hour of exposure. During the course of exposure to the lowest concentration used, response consisted of pro- and retropulsive movements at varied intervals and an average of two mild circumscribed clonic seizures.

Most of the thiols were irritating to the mucous membranes within approximately 15 minutes after exposure of animals to high concentrations as evidenced by their rubbing of the eyes and nose, eye closure, occasional sneezing, watering of the eyes, and retracting of the head. These signs were not observed in animals exposed to methyl heptanethiol. Corneal opacities or cloudiness in the eyes often occurred in mice just prior to or after death from exposure to propane-, butane-, and benzenethiol and occasionally ethane- and hexanethiol. Rarely was this condition seen in rats.

Inhalation LC_{50} values were not calculable for some of the thiols. Sufficiently high concentrations of alpha-toluenethiol vapors could not be obtained because of heavy condensation which occurred in the chamber and inlet tubes.

Ocular Exposure. The signs of eye irritation observed during inhalation exposures and the marked toxicity of benzene- and methyl heptanethiol via all routes of administration indicated the desirability of determining the local effects of thiols on the eye as well as possible systemic effects of the two above mentioned by this route. Each of the compounds tested, with the exception of hexanethiol, produced irritation of eye mucosa, although slight to moderate for all but benzenethiol. The latter not only produced severe irritation of the conjunctivae but also proved injurious to the cornea. The instillation of 0.1 ml of benzenethiol into the conjunctival sac of one eye of each of three rabbits produced moderate to severe redness, chemosis and discharge for three to four days

post-treatment. These conditions gradually subsided until final clearance of the conjunctivae on the 10th, 14th and 16th days. Corneal opacities, diffuse but involving three quarters of the cornea, developed in two rabbits on the fourth day and gradually increased in density to the 16th and 19th days, when opalescent areas covered details of the iris and caused difficulty in discerning pupil size. All evidence of corneal injury disappeared at 1.5 months following treatment of one rabbit and two months for the other. The treated eye of the third rabbit showed the same degree of corneal opacity as did the others, but subsided after the seventh day so no sign of injury persisted three weeks post-instillation. Injury from benzenethiol occurred to an even greater degree in rabbits whose eyes had been flushed with water. Eye conjunctivae of two animals showed signs of irritation until the 16th and 21st days post-treatment and corneal injury in the form of opacity developed to the fullest extent, i.e., opalescent areas completely obscured the iris at about three weeks in one rabbit while the other showed slightly less injury. These conditions persisted several weeks prior to the advent of recovery, and ultimate clearing occurred in approximately four and three months. Solutions of 0.5 and 1% silver nitrate, which complexes with thiols, were tested as an eyewash with two rabbits which had been administered benzenethiol; significant differences could not be detected between washed eyes and those receiving benzenethiol alone. Flushing with 0.5% silver nitrate solution immediately after thiol instillation followed by copious amounts of water sufficient to remove the visible silver-sulfhydryl complex, however, afforded good protection from the harmful effects of the thiol. Two rabbits treated in this manner showed slight inflammatory reaction of eye conjunctivae which had disappeared by the seventh and ninth days, and only one showed corneal opacity, this being diffuse and involving less than one quarter of the cornea. At no time did benzenethiol, or any other thiol tested, produce symptoms of systemic toxicity in treated rabbits.

Propanethiol was the only other compound which produced a severe degree of irritation beyond 48 hours. Instillation of this material produced heavy discharge and rather severe redness of the palpebral conjunctivae at the 24- through 96-hour readings, and chemosis (swelling with lids about half-closed) appeared at 48 hours. These conditions, which were the same for eyes washed with water, gradually subsided until final disappearance on the eighth day, post-treatment.

Depilation frequently occurred in areas around and below the eyes of rabbits from contact with benzenethiol as a result of washing. This effect was prominent at 5 to 6 days post-instillation and persisted 2 to 3.5 weeks.

Percutaneous Application. The toxicity of benzenethiol and methyl heptanethiol via skin absorption was assayed because of the marked effects of these compounds relative to other thiols. Mortality data and LD_{50} values with confidence limits for rats, and estimated LD_{50} values for rabbits are presented in Table IV. It is seen that benzenethiol was approximately five times more toxic for both rats and rabbits than was methyl heptanethiol when administered percutaneously. The former compound is classified[27] as "moderately" toxic while the latter is "slightly" toxic by this route. As observed for other routes of administration, benzenethiol produced some latent toxicity in both species whereas methyl heptanethiol did not.

Symptoms of toxicity induced by cutaneous application were similar to those previously described. Prior to their onset, however, rats exhibited irritative responses characterized by "ground-pawing" movements and frequent attempts to scratch and bite their backs. Methyl heptanethiol induced pre-seizure effects characteristic of this material. Benzenethiol-treated rats often displayed tremors followed by a convulsion of short duration prior to respiratory depression and coma. Rabbits, on the other hand, did not exhibit the signs of irritation seen in rats but did undergo methyl heptanethiol-induced pre-seizure symptoms prior to onset of fatal clonic and tonic seizures.

In addition, these compounds produced an inflammatory reaction of the skin a few hours after application. The redness usually disappeared within 24 to 48 hours. Benzenethiol had a lesser depilatory effect than was observed in rabbits treated intraocularly with this material.

Pathology. Autopsies made on animals dying from acute doses of thiols administered intraperitoneally, orally and percutaneously usually did not show significant gross or microscopic tissue changes. Rats receiving methyl heptanethiol had petechial and occasional small focal hemorrhages of the lungs, apparently caused by the violent convulsive seizures elicited by this material. Animals surviving single near-lethal doses by the intraperitoneal and particularly the oral routes, and sacrificed at various times within 20 days post-treatment, frequently showed pathologic changes which, although inconsistent, were indicative of liver and kidney damage. Microscopic examination revealed occasional marked changes in the kidneys of rats administered ethane-, propane-, 2-methyl-1-

TABLE IV
Toxicity Data for Rats and Rabbits Following Single Cutaneous Applications of Benzenethiol and Methyl Heptanethiol

Dose mg/kg	Mortality at Hours After Treatment								
	Rat					Rabbit			
	0–2	2–4	4–8	8–16	72	0–8	24	48	72
Benzenethiol									
67						0/3	0/3	0/3	0/3
134	0/5	0/5	0/5	0/5	1/5	0/3	0/3	1/3	2/3
213	0/5	0/5	0/5	1/5	1/5				
269	0/10	1/10	1/10	1/10	2/10	3/3 (All dead 4 hrs.)			
339	2/5	3/5	4/5	4/5	4/5				
427	2/5	4/5	4/5	4/5	4/5				
538	4/5	4/5	5/5						
LD_{50} (mg/kg)			300			estimated to be 134			
Confidence Limits			236–384						
Methyl Heptanethiol									
213	0/5	0/5	0/5	0/5	0/5	0/2–	0/2	0/2	0/2
427	0/5	0/5	0/5	0/5	0/5	0/2	0/2	0/2	0/2
854	0/5	0/5	0/5	0/5	0/5	2/2			
1708	3/5	3/5	3/5	3/5	3/5				
3416	4/5	5/5							
LD_{50} (mg/kg)			1954			estimated to be 600			
Confidence Limits			1135–2238						

propane-, and 2-methyl-2-propanethiol. Predominant changes were: degeneration with swelling and some necrosis of the tubular epithelium, thickening of Bowman's capsule, and hyaline deposition in glomerular tufts. More often, these and other thiols produced only minor lesions with varying degrees of cloudy swelling of the tubules and hyaline casts in the lumina. In general, liver changes were characterized by lymphocytic infiltration, occasional necrotic foci with small hemorrhages, and varying degrees of fatty degeneration. Only rarely did tissue studies show significant pathologic conditions as the result of relatively small doses of thiols.

Single intraperitoneal injections of near-lethal doses of thiols did not significantly affect the kidneys of rats, but effects were somewhat more prominent in the liver. The histopathologic changes in the liver may be generalized lymphocytic infiltration of the portal spaces, varying numbers of necrotic foci, occasional small hemorrhages, and a mild to moderate degree of fatty degeneration. These conditions were more often noted in animals receiving injections of ethane-, propane-, 2-methyl-1-propane-, 2-methyl-2-propane-, and hexanethiol, but never in animals receiving methyl heptanethiol.

Repeated subacute injections of benzenethiol produced only minor lesions. Fibrous thickening of the spleenic capsule was noted, which appeared to be a result of repeated intraperitoneal irritation. In addition, enlargement of the spleen was a common observation. In some rats the tubules of the kidneys showed a mild degree of cloudy swelling with hyaline casts in the lumina, and hyperemia of the adrenal medulla was a constant finding in all rats. Spherical macrophage accumulations among liver cords were frequently seen but were of doubtful significance, inasmuch as they are occasionally seen in "normal" rats.

Repeated injections of methyl heptanethiol produced marked fibrous thickening of liver and spleen capsules, otherwise the changes in internal organs were within normal limits. Sections from the brains of rats given these injections revealed no changes of consequence. In the cortex there was dilatation of pericapillary spaces, presumably due to mild edema, and the pial blood vessels were slightly engorged. Some vaculolization and gliosis was encountered but no changes could be demonstrated in nerve cells.

Animals dying several hours after exposure to high vapor concentrations showed mild to severe hyperemia of the trachea and lungs. Those dying from inhalation of methyl heptanethiol usually had petechial hemorrhages in the lungs. Moderate to near-lethal exposures to any given compound produced greater effects in mice than in rats. Characteristic findings,

varying in degree of severity but most prominent in mice exposed to butane-, hexane- and benzenethiol, were: liver changes consisting of cloudy swelling and fatty degeneration as early as 18 hours post-exposure, and necrosis which occasionally covered large areas; lung changes consisting of capillary engorgement, patchy edema and occasional hemorrhage; kidneys showed varying degrees of cloudy swelling, but more often mild to moderate; and rarely, benzenethiol-exposed mice showed hyperplasia of the spleen. Tissue studies of rats revealed essentially the same type of pathologic conditions as did mice, except for a high incidence of acute pneumonia. This was especially noted for benzenethiol-exposed rats in which emphysema was a constant finding. Hyperemia varied greatly and in some rats patchy edema was found but usually associated with pneumonia.

Histopathologic findings in animals exposed to thiol vapors, particularly those of benzenethiol, raised the question as to whether there was a true increase of pneumonia as a result of inhalation of these vapors. Evidence that benzenethiol is capable of exacerbating latent respiratory infection in rats was obtained by histologic observance of pulmonary infection in 7 to 10 rats exposed to low concentrations of the thiol on three consecutive days, whereas none of nine controls showed this condition.

Rarely (usually benzenethiol) did tissue studies show significant pathologic conditions as a result of exposure to minimal concentrations of thiols.

Comments

The general signs of acute thiol poisoning exhibited by mice, rats and rabbits were uniform for all compounds, except methyl heptanethiol (tertiary-octyl mercaptan), and were indicative of central depression and respiratory paralysis with death ensuing from respiratory failure. This pattern of response bears certain resemblance to that described by various investigators for hydrogen sulfide and certain other thiols.[9, 10, 13, 14] It seems odd, however, that the characteristic sedative action of the thiols noted in the present study has not been given more attention in the literature. Rats receiving maximal, sub-lethal doses of ethane- and butanethiol (occasionally propanethiol) intraperitoneally, and hexanethiol orally, showed especially notable sedation which approached anesthesia. The course of action involved the development of a reversible central depression leading to heavy sedation. Associated with the central depression was paralysis of the respiratory musculature, a critical factor in survival. If the animal survived the paralysis, a depression of relatively long duration ensued.

The finding that certain thiols produced sedative effects of greater degree by one route than another may be attributed to differences in rates of absorption, detoxication and excretion. In this connection, the even-numbered carbon, straight-chain members (C_2, C_4 and C_6) were significantly more toxic by all routes than were the 3-carbon straight-chain member (propanethiol) or the 3-carbon methylated members (2-methyl-1- and 2-methyl-2-propanethiol). Furthermore, relative toxicities decreased in the order of ethane-, butane- and hexanethiol for intraperitoneal and oral routes, whereas by inhalation the order of toxicity was practically reversed. The noted reversal in toxicity would appear to be predominantly influenced by absorption and elimination rates for the different routes of administration. Significant amounts of thiols were eliminated by exhalation as evidenced by characteristic thiol odors strongly detectable in the expired air of animals dosed by all but the ocular route. Rabbits injected intraperitoneally with 2-methyl-1-propanethiol (200 mg/kg) showed mean values of thiol in expired air of 0.18, 0.16 and 0.09 mg/minute/kg body weight at 30, 60 and 90 minutes, respectively, post-injection, indicating a rapid elimination of this thiol through the lungs. Values decreased to 0.03 mg/minute at the sixth hour, but perceptible amounts of thiol persisted as long as 24 hours post-injection. Pulmonary elimination was thus a significant factor in determining the ultimate toxicity and associated responses observed for this thiol; other thiols are presumably eliminated by this route at a greater or lesser rate.

The role of detoxication in thiol response, although important, has not been studied here and studies that have been made by others have been confined to methane- and ethanethiol. Canellakis and Tarver[28] reported that the sulfur in methanethiol administered to rats intraperitoneally appeared as sulfate in the urine and was not significantly incorporated into the methionine or cysteine of liver protein. According to Snow[29], sulfur of ethanethiol was excreted by mice and guinea pigs mainly as inorganic sulfate, but organic metabolites, one of which was ethyl methyl sulfone and one unidentified product, accounted for 10 to 20% of the sulfur excreted in the urine. The author postulated that methylation and oxidation converts ethanethiol to the sulfide and then to the sulfone. It is not known whether the methylation of ethanethiol, and other thiols, if they are metabolized in this manner, would interfere with normal methyl metabolism in rats. If so, it is conceivable that this could

account for some of the latent toxicity we have seen for all thiols except methyl heptanethiol. Snow, however, claimed no evidence of latent toxicity when compounds related to ethanethiol were administered to mice and guinea pigs, although he points out that the sensitivity of these species to interference of normal methyl metabolism is uncertain.

The effect of CNS stimulation of methyl heptanethiol alone, of all the thiols tested, was striking. The stimulatory effects were typical of other well known CNS stimulants such as picrotoxin and pentylenetetrazol (metrazol). Methyl heptanethiol appeared to act at various levels of the cerebrospinal axis, and apparently at a higher level than that of strychnine, inasmuch as the convulsive seizures induced by methyl heptanethiol were spontaneous in origin and not "triggered" by external stimuli as is known in the spinal convulsions induced by strychnine. Typical of CNS stimulants, methyl heptanethiol had an analeptic action on the higher CNS centers. Evidence for this was found in sub-convulsant doses, wherein methyl heptanethiol strongly stimulated the respiratory and vasomotor centers. This analeptic action was strikingly demonstrated by the capacity of this thiol to counteract the depression produced by hypnotic drugs such as barbiturates. When the convulsant thiol was used after depressant doses of barbiturates, the depressed pulse and respiration rates could be restored to levels even above normal. Conversely, barbiturates were found to counteract the toxic action of methyl heptanethiol by preventing the convulsive seizures it induced. It would appear, therefore, that barbiturates and/or other central depressant hypnotics would be the rational therapy of poisoning by this thiol. This phase of study, together with that concerning the analeptic action of methyl heptanethiol against the depression of hypnotics, is to be reported in a separate publication.

It should be noted, however, that despite the opposite CNS action of methyl heptanethiol, the end result of acute poisoning was similar to that produced by maximal lethal doses of other thiols; excessive CNS stimulation produced by methyl heptanethiol was followed by central depression, then death as a result of respiratory failure. This striking contrast in action observed for members of homologous series of compounds is rare, but by no means unknown.[30, 31]

Another finding of particular interest was the antagonism of the convulsant methyl heptanethiol toward the depressant action of the other thiols. Antagonism was observed only for minimal lethal doses, however; methyl heptanethiol was not able to counteract the depressant and paralytic action of thiols at higher doses, presumably because at required higher doses a threshold level was attained whereby the ultimate depressant action of methyl heptanethiol potentiated rather than antagonized the effects of the thiols being counteracted. Interactions of this partial antagonism are to be investigated further.

The CNS stimulatory effect is apparently unique for methyl heptanethiol not only among compounds possessing thiol structure, but also among compounds containing either the same or closely related hydrocarbon structure. Neither n-octyl, tert-hexyl and tert-dodecyl mercaptans, nor tert-octyl alcohol, or tert-octyl amine, gave evidence of stimulatory action.

From the standpoint of acute toxicity, thiols, generally, with the exception of benzene- and methyl heptanethiol, were found not to be particularly toxic (Table V). Classification of the acute toxicity of thiols from the combined tabulated data for mice, rats and rabbits for all routes tested shows that benzene- and methyl heptanethiol are the only compounds rated as "highly" toxic. Other compounds ranged in toxicity from "moderately" toxic to "practically nontoxic."

A particular distinction should be made, however, between the above toxicity rating of the thiols and their hazards. On a hazard basis, ethane-, butane-, hexane- and benzenethiol would be rated moderately severe to very severe by inhalation by virtue of their high volatility, inhalation LC_{50} values, and their suspected role (demonstrated for benzenethiol) in exacerbating latent respiratory infection.

It has been repeatedly observed during the course of the inhalation portion of the work that animals frequently died, not from exposure to the thiol per se, but from an associated pneumonia. Because secondary infection usually did not follow exposures to low concentrations (with the exception of benzenethiol), it is assumed that concentrations sufficiently high to be irritating to the respiratory tract must obtain in order to favor the growth of microorganisms. This irritative response, coupled with a long period of lethargy and its associated shallow respiration, would appear to favor as well the fatal issue of a hypostatic pneumonia and/or other pulmonary complications. These secondary complications of thiol exposure have obvious industrial health significance.

Although methyl heptanethiol was found not to elicit significant respiratory irritation with resultant latent infection, its high toxicity by all but the ocular route would qualify it as a severely hazardous material. The inhalation hazard of alpha-toluenethiol, because of the low volatility

TABLE V
Combined Tabulation of Acute Thiol Toxicity for Various Routes of Administration and Animal Species

Compound	IP LD_{50} mg/kg	Oral LD_{50} mg/kg	Inhal. LC_{50} ppm		Eye Irritation	Skin LD_{50} mg/kg		Toxicity Class[a]
	Rats	Rats	Mice	Rats	Rabbits	Rats	Rabbits	
Ethanethiol CH_3CH_2SH	226	682	2770	4420	Slight			Slightly
Propanethiol $CH_3(CH_2)_2SH$	515	1790	4010	7200	Moderate			Slightly
2-Methyl-1-propanethiol $(CH_3)_2CHCH_2SH$	917	7168	>25000[b]	>25000[b]	Very Slight			Practically Non-toxic
2-Methyl-2-propanethiol $(CH_3)_3CSH$	590	4729	16500	22200	Slight			Practically Non-toxic
Butanethiol $CH_3(CH_2)_3SH$	399	1500	2500	4020	Slight			Slightly
Hexanethiol $CH_3(CH_2)_5SH$	396	1254	528	1080	None			Slightly
Methyl heptanethiol $C_8H_{17}SH$	12.9	83.5	47	51	Slight	1594	600	Highly
Benzenethiol C_6H_5SH	9.8	46.2	28	33	Severe	300	134	Highly
Alpha-Toluenethiol $C_6H_5CH_2SH$	373	493	178	>235[b]	Slight			Moderately

[a] Toxicity classification of Hine & Jacobson.[37]
[b] LC_{50} not calculable.

of this compound, would appear to be considerably less than that of the above mentioned compounds. In addition, propanethiol would appear slightly hazardous, whereas 2-methyl-1- and 2-methyl-2-propanethiol would be rated as relatively nonhazardous because of their high LC_{50} values.

Personnel handling thiols under circumstances which might involve spillage should be warned against breathing the contaminated atmosphere for prolonged periods. This holds especially for benzene- and methyl heptanethiol which would undoubtedly produce serious effects if near-saturated concentrations were inhaled *even for short periods*. The toxic effects of benzene- and methyl heptanethiol by the cutaneous route further emphasize the need for exercising care in handling in order to avoid spillage and subsequent contamination. In this event, there is, in addition to skin absorption, the hazard of inhaling rebound vapors.

Particularly pertinent to possible skin exposure hazards, especially from the longer chain thiols, is the recently reported study by Brooks et al.[32] wherein it was shown that certain mercaptans (n-dodecyl and n-octadecyl) were capable of producing epidermal hyperplasia in mice. The suspected relation between hyperplastic agents and carcinogens or cancer-promotors makes this finding of special significance here.

The irritative effects, observed in the eyes of rabbits following intraocular application of certain thiols (Table V), indicate a significant hazard to eyes from splashing liquid. Relatively small volumes of all but hexanethiol can produce at least moderate irritation of the conjunctivae and benzenethiol can cause severe corneal damage with resultant visual handicap. Contaminated eyes should be washed thoroughly and promptly with a weak silver nitrate solution (0.5%) or some other complexing agent (silver antiseptic such as argyrol) and then flushed with copious amounts of water.

The rationale in emergency treatment of general thiol intoxication indicates the usual supportive therapy for terminating the effects of central depressants, e.g., maintenance of a patent airway, the administration of oxygen and/or use of artificial respiration. The noted antagonism observed between methyl heptanethiol and other thiols suggests that analeptic agents may be of some value in counteracting the toxic action of thiols, but obviously contraindicated for CNS stimulants such as methyl heptanethiol.

If some of the thiols reported here appear to be unimportant toxicologically as components of urban air pollution, there still remains the real possibility of their oxidative products being of considerable importance,[35] or the thiols themselves may prove to be of air pollution concern for as yet unrecognized reasons. The potential hazards of benzene- and methyl heptanethiol as air pollutants would appear significant not only because of their intrinsic high toxicity, but also for their as yet unknown cumulative effects at air pollution levels.

Summary

Toxicity data are reported for seven aliphatic and two aromatic thiols administered intraperitoneally and orally to rats in single acute doses, by single, 4-hour inhalation exposures to both rats and mice, and by eye instillation in rabbits. In addition, two compounds were administered percutaneously to rats and rabbits.

Compounds tested, and listed in order of decreasing toxicity were benzene-, methyl heptane-, alpha-toluene-, ethane-, hexane-, butane-, propane-, 2-methyl-2-propane-, and 2-methyl-1-propanethiol. Results for all but the ocular route are given as LD_{50} and LC_{50} values calculated for various periods up to 15 days post-administration.

A classification of toxicity based on results obtained for all routes of administration rated two compounds, benzene- and methyl heptanethiol, as having a high degree of toxicity and lethality. The two least toxic compounds, 2-methyl-2-propane- and 2-methyl-1-propanethiol, were rated as practically nontoxic, whereas the remainder ranged from moderately toxic to slightly toxic.

Thiols had the common toxicologic property of being soporific, the degree ranging from mild stupor to heavy sedation. Methyl heptanethiol was an exception, producing a powerful CNS stimulatory effect. Relatively deep sedation was produced by maximal, sublethal doses of ethane-, propane-, butane-, and hexanethiol. Certain thiols produced a high incidence of *latent* mortality. The associated responses of thiol toxicity varied according to the route of administration.

Significant amounts of thiols were eliminated in the expired air of animals dosed by all but the ocular route. Thus, pulmonary elimination of thiols was a factor of importance in determining ultimate toxicity of these materials.

Acute thiol poisoning produced a uniform pattern of central depression and respiratory paralysis with death ensuing from respiratory failure. Methyl heptanethiol, on the other hand, was typical of well-known CNS stimulants such as picrotoxin and pentylenetetrazol having an analeptic action on the higher CNS centers and producing motor, respiratory, and vasomotor stimulation in relatively small doses. Methyl heptanethiol was shown to counteract the central depression induced by barbiturates. Antagonistic action was reciprocated in that barbiturates were found to inhibit methyl heptanethiol-induced CNS activity. Methyl heptanethiol also showed antagonism toward the depressant action of other thiols, but was unable to counteract the toxic effects of maximal lethal doses. Toxic sublethal or lethal doses of methyl heptanethiol induced clonic and/or tonic seizures ending like other thiols in central depression, respiratory failure and death.

Repeated intraperitoneal injections, three per week for 3 and 4 weeks, of benzene- and methyl heptanethiol, respectively, at approximate $\frac{1}{8}$ LD_{50} doses were tolerated by rats; the latter compound, however, produced mild convulsive seizures.

Splenic enlargement was the only gross pathologic change produced with consistency. Benzenethiol produced injury to the cornea and conjunctivae of rabbits and lethal doses of methyl heptanethiol frequently produced petechiae in the lungs. Thiol-exposed rats and mice commonly showed latent pulmonary infection and/or pneumonia. Liver and kidney damage occurred from intraperitoneal inhalation, and particularly oral administration, but at relatively high levels. Inhalation at high levels produced slight to severe irritation of the respiratory tract, the degree dependent on the thiol; benzenethiol was the only compound to produce significant pathologic conditions at lower levels of thiol exposure.

Safety precautions are given for conditions wherein personnel might be exposed by accident to the more toxic thiols and/or their vapors, and measures are suggested for emergency treatment of thiol intoxication.

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JOHN J. BLOOMFIELD (center), recipient of the Donald E. Cummings Memorial Award for 1958, accepts the certificate of award from retiring President, Charles R. Williams. Incoming President, Kenneth W. Nelson (right) expresses his approval. The Award was made at the annual banquet of the American Industrial Hygiene Association, April 22, 1958, in Atlantic City.

The Acute Toxicity of Intramuscular Parathion in Rats and the Relation of Weight, Sex and Sex Hormones to this Toxicity

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Parathion, also known by a host of other names, is a powerful organic phosphate insecticide. Its acute toxicity is high and it exerts a strong cholinergic action in mammals. This cholinergic action is accredited to its inhibition of the enzyme cholinesterase. This is supported by the fact that the symptoms produced are typical of parasympathetic stimulation, and because atropine, which inhibits the action of acetylcholine, offers protection against some of its action.

Many studies of parathion toxicity have been made on the rat. These have included acute and chronic values for parathion by oral, intraperitoneal, intravenous and dermal administration. Most of the studies have dealt with oral administration, however, parathion has been found to be rapidly absorbed and highly toxic by dermal application as well as by ingestion. Although intramuscular injection would be a means of quantitating dermal absorption, no intramuscular studies have been reported.

The LD_{50} values for parathion in the rat have been recorded by many investigators.[1-6] The values differ from investigation to investigation even when the routes of administration were identical. In the instances where both sexes were used, the female rat was found to be more susceptible than the male when parathion was given by the oral or intraperitoneal route. The literature does not contain any studies of intravenous, dermal or intramuscular administration of parathion where comparisons were made between the sexes. Since no intramuscular LD_{50}'s have been reported, the first purpose of this investigation becomes one of establishing intramuscular LD_{50}'s for young mature (140 to 220 gm) male and female rats. If a difference between the sexes is found by intramuscular administration, then a similar sex difference could be predicted for dermal or intravenous administration.

DuBois *et al.*[1] reported no difference in the susceptibility of mature (300 gm) and immature (150 gm) male rats. A 150-gram male rat is hardly immature and if smaller rats are used there might be a difference in the susceptibility between immature and mature males and this might also hold true for female rats. Thus, the second purpose becomes one of establishing if there is a difference in the susceptibility to parathion for rats of different weights.

The sex difference in response to parathion in rats has largely been attributed to the greater susceptibility of the female. Since parathion is known to inhibit cholinesterase, investigators have tried to demonstrate a relationship between the activity or the inhibition of the enzyme and the sex of the animal. This has not been accomplished.

There have been attempts to establish a relationship between the enzyme and the sex hormones. Even these do not explain the difference. In an attempt to establish the reason for the sex difference, DuBois *et al.*[1] injected rats with the opposite hormones and reported that this tended to equalize their susceptibility to the parathion. These experiments have been repeated and extended with the possibility of adding new information on the sex differences of rats to parathion.

Methods

The parathion used in these experiments was obtained from the American Cyanamid Co. of New York. The parathion was of 99.7% purity and its specific gravity was 1.27. Parathion is almost insoluble in water but is soluble in organic solvents and generally is used in propylene glycol. Hazleton and Holland[2] have shown that in either sex a propylene glycol solution

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of parathion is more toxic than the wettable powder in aqueous suspension. In preliminary tests, propylene glycol was found to be very irritating to the rat when injected into the muscle. Of the solvents tested, peanut oil was found to produce the least attempts to bite and squirm. Therefore, a standard stock solution was made up using a weighed amount of parathion and diluted volumetrically with peanut oil. From the standard solution twenty-one aliquots were made in logarithmic order from 5.4 mg/ml to 52.9 mg/ml. The animals received 0.1 ml/100 gm of body weight. Thus, the amount of the solution injected remained constant with the weight of the rat because as the concentration of parathion increased the amount of peanut oil decreased. This method was used so that the volume injected would not be too great for the amount of muscle tissue.

Injections were made through the biceps femoris into the semimembranosus or semitendinosus muscles. The syringe used was a ¼ ml tuberculin with 0.005 ml graduations. The needle was 23 gauge with ½ inch length.

The rats used in these experiments were colony inbred albinos of the Wistar strain. Each group of males or females consisted of ten animals with the exception that in the castration and ovariectomy experiments the groups were composed of five rats. Three hundred rats in the weight range of 140 to 220 gm were used to establish the LD_{50} for the mature rats of the size generally used for toxicity studies. Then, after the LD_{50} for the mature rats was established, rats of lesser and greater weights were used to establish LD_{50} values in reference to the differences in sex susceptibility with regard to weight. One hundred rats were used in each weight group. They were sub-divided into groups as follows: (1) 50 to 60 gm or weaning size, (2) 70 to 80 gm or pre-pre-puberty, (3) 90 to 120 gm or pre-puberty, (4) 240 to 300 gm or breeders and (5) 310 to 400 gm or old breeders. Only males were available in the latter group. No rats were included in the puberty size with weights between 120 and 140 gm as they were used for castration and ovariectomy. In the hormone injection experiments, young mature adult rats in the weight range of 140 to 220 gm were used.

TABLE I
Evaluation of Sex and Weight Differences with Dose-Mortality Reaction in Rats

Weight Group (grams)	Sex	LD_{50} (19/20 C.L.)
50 to 60	Male	11.5 (9.8–13.7)
	Female	10.5 (8.8–12.5)
70 to 80	Male	12.6 (10.5–14.5)
	Female	8.8 (7.3–10.6)
90 to 120	Male	13.9 (11.8–16.3)
	Female	13.4 (11.4–15.7)
140 to 220	Male	41.4 (37.9–45.4)
	Female	10.4 (9.3–11.6)
240 to 300	Male	41.8 (36.1–48.5)
	Female	11.4 (9.2–14.0)
310 to 400	Male	45.0 (38.2–53.1)

The observations on the number of deaths were made as follows: (1) up to 4 hours after injection, (2) 4 to 24 hours after injection, (3) 24 to 48 hours after injection, (4) 48 to 72 hours after injection. All surviving animals were observed for 96 hours. However, no animals died beyond the 72-hour interval. The dose-mortality data were analyzed by the method of Bliss[7] and the LD_{50} values with confidence limits are presented in Table I.

The hormone effect on parathion toxicity was studied by experiments which included the injection of the same and opposite hormone into normal rats and rats with castration or ovariectomy. Ten rats were used in each group of normal animals that were injected with hormones. The design of the experiment allowed for ten rats of one sex or the other in all groups. However, due to a change of the project before the operations were completed, the groups of rats with castration or ovariectomy contained only five animals of each sex.

The hormones used in these experiments were estrone obtained from Abbott Laboratories and testosterone propionate obtained from Daltone Chemical Co. The total dose of hormone, 6 mg/kg, was given in single doses of 1 mg/kg of body weight for six days. On the seventh day the rats were injected with parathion. The experimental design allowed for the inclusion of multiple dose levels. At the present stage of work only three levels have been utilized. These levels were purposefully selected. The low level dose was at the female LD_{50} value. The middle level was 21.5 mg/kg where all of the females died but none of the males died. The upper level was at the LD_{50} value for males.

The operations were performed when the rats were in a weight range of 120 to 140 gm. The rats were allowed to recover for three weeks then the hormones were injected for a week and followed by the parathion injections. Injection of hormones was into one leg and parathion into the other. The data on the injections into non-operated and operated animals were

TABLE II

Evaluation of Sex Hormones with Dose-Mortality Reaction in Rats

Non-operated		Operated	
Group	LD_{50} (19/20 C.L.)	Group	LD_{50} (19/20 C.L.)
Control female	10.4 (9.3–11.6)	Ovariectomy, female without hormone	11.1 (6.5–19.0)
Female with estrone	<11.6	Ovariectomy, female with estrone	—
Female with testosterone	15.3 (7.5–31.4)	Ovariectomy, female with testosterone	15.8 (zero range)
Control male	41.4 (37.9–45.4)	Castration, male without hormone	21.4 (15.1–30.4)
Male with estrone	16.6 (11.4–24.0)	Castration, male with estrone	19.1 (13.5–27.2)
Male with testosterone	29.7 (23.0–38.4)	Castration, male with testosterone	>36.9

analyzed by the moving average method of Thompson,[6] as there were five animals in some groups and ten in others and only three dose levels were used. The LD_{50} values are presented in Table II.

Results and Discussion

TIME-MORTALITY:

The first experiment was conducted upon the 140 to 220 gm rats in order to establish the intramuscular LD_{50} for young mature animals of both sexes. Then experiments on rats of other weight ranges were added for comparisons in regard to weight. Although only the LD_{50} values are presented in Table I, the deaths were recorded for 24-hour periods to show approximately when death occurred. The first period was further divided into a 4-hour period and a 20-hour period to illustrate how many animals died immediately.

The deaths ranged in time from ½ hour to 72 hours. However, very few animals died beyond 48 hours. The greatest number of deaths occurred between 4 and 24 hours. Hazleton and Holland[2] stated that, depending on the dosage and preparation, death may occur from one to 24 hours. Also, animals that survive more than 24 hours may be expected to recover and there is no apparent latent toxicity. In the present study a time cumulative per cent mortality curve for the averages of male and female rats is presented in Figure 1. An equal percentage of both males and females died within the first four hours; whereas, at 24 hours 56% of the females were dead and only 28% of the males had died. There was little, if any, increase in female deaths after 24 hours, whereas, the males died at a uniform rate from 24 to 72 hours.

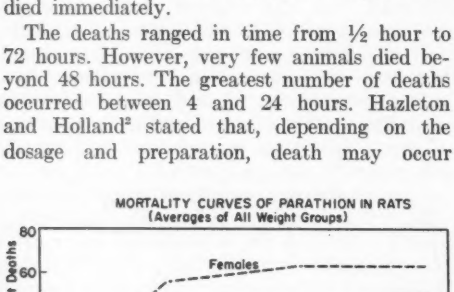

FIGURE 1

DIFFERENCES IN SEX AND WEIGHT:

The LD_{50} values and confidence limits for males and females of all weight ranges are presented in Table I. In order to illustrate the differences between weight and sex, these values are presented graphically in Figure 2. The LD_{50} value for the 140 to 220 gm female rat is 10.4 mg/kg of body weight. The value for the male rat of this weight range is 41.4 mg/kg of body weight. The 240 to 300 gm males and females have LD_{50} values similar to the 140 to 220 gm animals. The immature males (up to 120 gm) and all females have no significant difference in their LD_{50} values. Thus, the difference between immature males and mature males is about the same as between mature males and all females. DuBois *et al.*[1] have stated that there is no observable difference in the susceptibility of immature male rats 150 gm and mature male rats 300 gm to parathion. The present study agrees with this finding between 150 and 300 gm males but the results indicate that 150 gm males are not immature, as there is a great difference in susceptibility between male rats of 50 to 120 gm and male rats of 140 to 400 gm.

The results indicate that the female does not become more susceptible but that the male obtains added protection when sexual maturity is reached. Thus, testosterone would seem to have some role in this protection. The question is then raised as to how this is accomplished and what mechanisms are involved.

The mechanism of action in parathion poisoning is generally thought to be one of cholinesterase inhibition. This is based on the evidence that parathion inhibits the activity of the enzyme in the body and that acute toxic doses produce symptoms which are similar to excessive stimulation of the parasympathetic and central nervous systems. In the present experiments when the progression of death was slow enough so that symptoms could be observed, they occurred in order from irritability to fibrillary twitching of the leg muscles, then lacrimation, urination and sometimes defecation, severe muscular contraction followed by tonic and later clonic convulsions with a final arching of the tail (opisthotonus) preceding death. The last finding has not been previously reported for parathion. However, this is typical of excessive central nervous system stimulation as in strychnine poisoning. Death appears to be due to this excessive central nervous system stimulation which parallels and possibly exceeds the parasympathetic stimulation.

The mechanism of action by which parathion produces death does not explain why mature males are less susceptible than females or immature males. Some investigators have attempted to explain the sex difference as being due to some characteristic in the female rat that renders her more susceptible than the male. Since parathion functions as a cholinesterase inhibitor, an explanation for the sex difference was looked for in terms of the enzyme. Prior to the discovery of a sex difference to parathion, Beveridge and Lucas[9] had shown that the female rat exhibits greater cholinesterase activity than the male. Investigators hoped to demonstrate that the cholinesterase of the female undergoes a greater degree of inhibitation. However, this has not been established.

There have been attempts to establish some relationship between the enzyme and the sex hormones. Torda[10] found that estrogens slightly depressed the activity of specific cholinesterase in vitro and stimulated acetylcholine synthesis while androgens depressed it. Sawyer and Everett[11] also stated that estrogens do not affect the activity of cholinesterase directly but stimulate synthesis of the enzyme. They have shown that the plasma cholinesterase

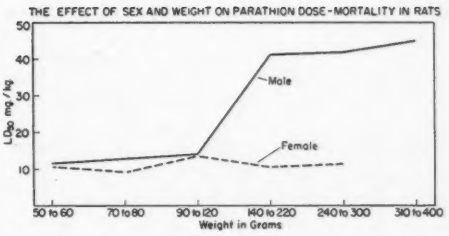

FIGURE 2

level appears to parallel the plasma estrogen level. In a later article[12], they have demonstrated that the amount of non-specific cholinesterase in rat plasma is controlled, at least in part, by sex hormones. Estrogens elevated the non-specific enzyme and testosterone depressed it, while progesterone exerted no noticeable effect except indirectly through estrogen. These conditions led to the marked difference in plasma cholinesterase levels of the mature rats with the female having several times as much enzyme in the plasma as the male. Birkhauser and Zeller[13] have demonstrated a corresponding sex difference in liver cholinesterase activity. While the evidence establishes some relationship between the hormones and the enzyme level, it does not offer an explanation for the difference in susceptibility.

HORMONE INJECTIONS:

In an effort to find an explanation for the sex difference in the susceptibility of rats to parathion, DuBois *et al.*[1] injected twelve male rats with diethyl stilbestrol and twelve female rats with testosterone propionate prior to injection with parathion. The calculated LD_{50}'s indicate that the difference in susceptibility between the male and female rats may be removed by the injection of the opposite hormone. This finding demonstrates that there is a connection between the sex hormones and the difference in the susceptibility of male and female rats to parathion. However, their work does not demonstrate if there is an antagonistic or synergistic action between the injected hormone and the normal hormone, which might explain the change in the susceptibility of the male and female to parathion when the opposite hormone is injected. These experiments were repeated and extended in the present study. Not only were the animals injected with the opposite sex hormone but the female rats were also injected with female hormone and male rats were injected with male hormone. Also rats with cas-

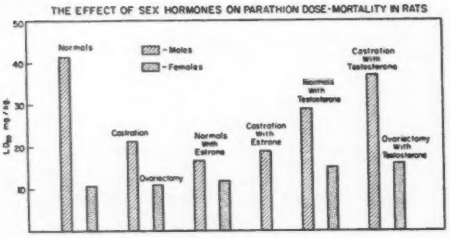

FIGURE 3

tration or ovariectomy were injected with hormones.

The mortality data of the hormone injections were analyzed by the moving average method of Thompson.[8] The LD_{50} values are presented in Table II. These values are presented graphically in Figure 3 so that comparisons can be made quickly.

1. Female vs ovariectomized female:

There is no change in the mortality of the females even with ovariectomy until testosterone is given. The LD_{50} values of the ovariectomy female without hormone injections and the female with estrone injections fall within the confidence limits (9.3–11.6 mg/kg) of the control female. The value for the female injected with testosterone and the ovariectomy female injected with testosterone were 15.3 and 15.8 mg/kg respectively. Thus the injection of testosterone into females with or without ovariectomy gives them added protection.

2. Male vs castrated male:

The changes in the male with the injection of hormones into normal and castrated rats is marked. The LD_{50} value of the control male is 41.4 mg/kg and the confidence limits are 37.9 to 45.4 mg/kg. The male loses about one-half of his protection with castration as indicated by a fall of the LD_{50} value to 21.4 mg/kg. When the castrate is injected with testosterone, the value returns to 36.9 or just below the confidence limit of the controls. Thus, the castrate regains most of his original protection with the injection of testosterone. There is a possibility that the injection of testosterone is not sufficient to maintain the level of androgen in the blood so that the same protection could be obtained as in the control male. The LD_{50} value of the castrated male is reduced from 21.4 to 19.1 with the injection of estrone. The value for the normal male when injected with estrone falls to 16.6 mg/kg. When injected with testosterone, the normal male also has a decrease in the LD_{50} value to 29.7 mg/kg. Thus there is an increase in susceptibility or a loss in protection by the male when injected with testosterone. The reason this occurs cannot be definately established as hormone levels of the blood or urine were not determined. However, it is known that when a target gland hormone is given in heavy doses and suddenly discontinued, the function of the target gland is decreased—sometimes to a severe degree for several days—and then returns to normal.[14] This would indicate that the injected testosterone in the normal males could elevate the androgen level of the blood to a degree which suppresses the gonadotropic stimulating fraction of the anterior pituitary. Thus, the testicular androgen production would decrease and the blood level would fall below normal. If the parathion is injected at this time and if the protection is dependent upon the level of androgens in the blood, then this would explain the slight increase in susceptibility. Injected estrone may act in the same manner in the male as the injected testosterone in the male since estrone alters the protection of the normal male but does not affect the castrated male as much. However, the difference is not great. Further experiments are necessary to clarify these points.

These experiments do not fully answer whether injected estrogens and androgens act antagonistically to the normal hormone in changing the susceptibility of male rats to parathion. However, they demonstrate that the male has protection and that the injection of hormones lowers this protection. Castration does not produce as great a loss of protection nor does the injection of estrogen into castrated males cause as great a change. The injection of testosterone into the normal female or the female with ovariectomy gives protection. Also injected testosterone into castrated males gives them added protection over normal males with testosterone. How the androgen acts and how the injected estrogen acts in relation to the parathion cannot be definitely stated at this time. However, the injection of substances which stimulate testosterone production may increase the protection even more.

Summary

The intramuscular LD_{50}'s to parathion have been determined for a large number of young mature male and female rats in the weight range of 140 to 220 gm. These values are 10.4 mg/kg for the female and 41.4 mg/kg for the male. In order to determine if this difference holds for rats of other weights, intramuscular LD_{50}'s were also established for 50 to 60 gm, 70 to 80 gm, 90 to 120 gm, and 240 to 300 gm rats. The LD_{50}'s are, in order for female and male as follows: 10.5 and 11.5

mg/kg, 8.9 and 12.6 mg/kg, 13.4 and 13.9 mg/kg, 11.4 and 41.8 mg/kg. The immature male and all females have approximately equal values, while the mature male values are high. The results indicate that the male obtains added protection to the toxicity of parathion when sexual maturity is reached.

In order to determine what effect the sex hormones have on the toxicity of parathion, intramuscular injections of the same and opposite sex hormones have been made into normal rats and rats with castration or ovariectomy. The LD_{50} values were determined for the non-operated rats with and without injected hormones. The values are as follows: female with female hormone, 11.6; female with male hormone, 15.3; male with female hormone, 16.6; and male with male hormone, 29.7. Thus, male hormone in the female gives her added protection, and the female hormone into the male decreases his protection to parathion. The male hormone injected into the male also decreases his protection. However, when male hormone is injected into castrated males, their protection is increased. The male hormone injected into the female rat with ovariectomy also gives her increased protection over normal or ovariectomy females. The female hormone injected into castrated males causes a slight decrease in protection over castrated males. Thus, the results of the injection experiments indicate that injected testosterone propionate offers protection to the female rat, to the castrated male and to the female with ovariectomy. The injection of estrone into the male decreases his protection. Castration of the male also decreases his protection and the injection of female hormone into the castrate causes further loss of protection.

The male sex hormone appears to have a major role in the added protection of the mature male rat to the toxicity of parathion. This may be related to the level of androgen in the blood since castration or the injection of estrone or the injection of testosterone decreases this protection by different degrees. How androgen acts and how other injected hormones may act in relation to the androgen or to the parathion cannot be definitely stated at this time. Further studies are necessary in order to determine the exact role of androgen or other hormones in the toxicity of parathion to rats.

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The Deposition of a Submicronic Aerosol in the Respiratory Tract of Dogs*

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Introduction

THIS experimental study is concerned with one phase of an investigation into the effects and fate of inhaled alpha emitters.[1, 2] Certain technical problems associated with handling trace quantities ($<\mu g/m^3$) of such radioisotopes in aerosol form compel the use of a vector or carrier aerosol. The aerosol utilized consisted of submicronic crystals of sodium chloride. The first studies with this sodium chloride aerosol were made in human subjects, in an attempt to reveal the role of some physiologic and physical parameters on the deposition of the aerosol in the human respiratory tract during uncontrolled respiration.[3]

Following the human studies, a series of studies were initiated with dogs which incorporated all of the features of the human study including the experimental apparatus and the same aerosol.

Because it was possible to contaminate the sodium chloride aerosol in the dog study with suitable amounts of alpha activity without appreciably affecting its properties, the dog study served two ends: first, as a fundamental study into the role of certain factors on dust deposition (comparable to the human study) and second, it provided animals with respiratory tract burdens of alpha emitters and thereby served as a component of the alpha emitter program.

Those aspects of the dog study which are concerned with the radiation effects, distribution, excretion and lung retention of the alpha emitter used, polonium^{210}, will be reported elsewhere.

Methods and Materials

The two basic functions of the apparatus used to measure deposition are: (a) to separate the inspired and expired airs in synchrony with the animal's breathing pattern, and (b) to measure the volumes of air and concentrations of dust breathed. Details of the equipment involved have been described previously.[2, 3, 4] The data obtained from the deposition apparatus are in three forms: (a) a pneumotachogram, (b) individually recorded and integrated tidal volumes (analog integrator), (c) a digital integrator output which is equivalent to the total air respired.

In Figure 1, two pneumotachograms and one analog integrator record are reproduced. A comparison of the two pneumotachograms demonstrate that even when the tidal volumes of two dogs are within 10 per cent of each other, there may be remarkable differences in their respiratory characteristics. From such recordings, it is possible to directly measure the tidal volume, respiratory frequency, minute volume, mean air flows, maximum air flows, phase durations, pause lengths, air flow accelerations and air flow decelerations.

The sodium chloride aerosol was produced by a jet aspirator[5] and had a count median diameter of 0.04 micron (σ_g 2.3) and a mass concentration of 10–30 mg/m^3; the latter was controlled by the amount of diluting air used. The aerosol generator chamber and samplers have been described elsewhere.[6, 7] All particle size analyses were made from projected electron photomicrographs (~100,000 ×).

The aerosol collected was analyzed by a flame-photometric method for sodium and by a titrometric method for chloride.[8] When alpha emitters were present, the alpha activity and the chloride was measured but not the sodium. In no instance did the sodium, chloride, and/or alpha activity measurements differ by more than ±5% of giving identical mass deposition values.

The dogs (females 6–12 kg) studied were premedicated with oral doses of secobarbital (~18 mg/kg) and chlorpromazine (~10 mg/kg). While the sedation produced was somewhat variable, in only one instance was an important respiratory depression noted. The dogs were generally sleepy and slightly ataxic. An accidental overdose of chlorpromazine produced a marked tachypnea, salivation and slight tremor in one dog. In later experiments, intravenous secobarbital was used without the tranquilizer. The dogs were slowly given a 10 mg/ml solution for a

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Portions of this study were presented at the American Industrial Hygiene Association Meeting, April 24, 1958, Atlantic City, N. J.

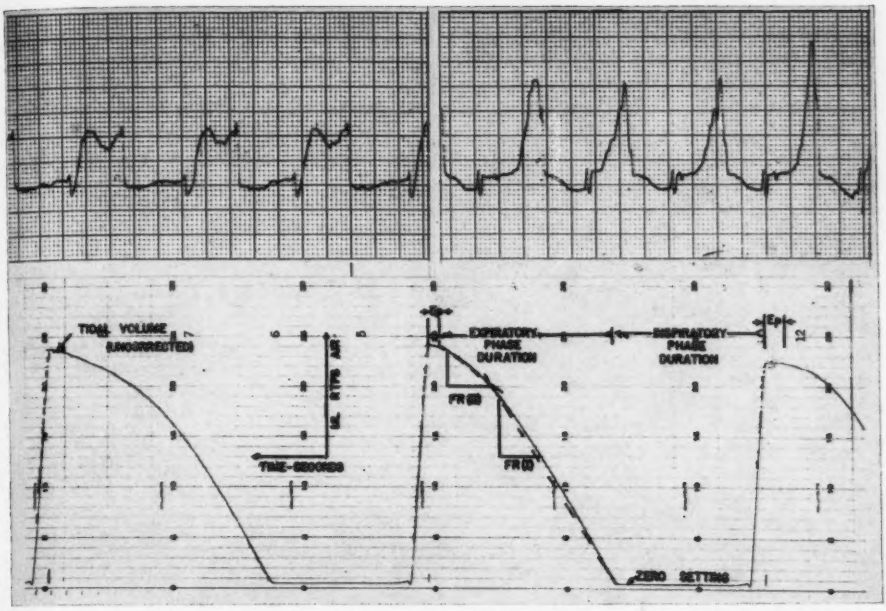

FIGURE 1. In the pneumotachograms (upper records) the ordinate is the expired air flow (ml/sec and the abscissa is time (sec). The area under the curves is proportional to the expiratory tidal volume In the left record, mean tidal volume, expired air flow and maximum expired air flow were 107 ml, 52 ml/sec and 90 ml/sec, respectively; in the right record, 119 ml, 52 ml/sec and 170 ml/sec, respectively.

In the integrator record (lower record) the abscissa is time (sec) and the ordinate is tidal air volume (ml RTPS air). The phase durations, expiratory pauses (EP) and mean expiratory flow rate (FR) are also indicated.

dose of from 3–5 mg/kg; thereby, a very consistent state of sedation without anesthesia was obtained.

During the experiments (20–40 minutes duration) the dogs were maintained in a modified Pavlov stand supported by a shoulder harness and an abdominal band. The head was held fixed in a natural position with the muzzle strapped into a specially fitted mask.

The estimation of the mass deposition was performed according to the following equations:

$$\% \text{ deposition} = 100 \times [1 - (E)/(I)]$$

and

$$\text{Absolute deposition} = \% \text{ depositn.} \times \text{I vol.} \times (I)$$

Where (I) and (E) are the inspired and expired air concentrations, respectively, and I vol. is the total volume of air breathed. The aerosol lost in the mask and valve airways was analyzed and this amount was added to expired air value before calculation of the expired air concentration (E).

Results

The mean deposition value from nineteen experiments on nine dogs was determined to be 66.5 per cent of the aerosol mass respired. Individual experimental values are portrayed in Figure 2.

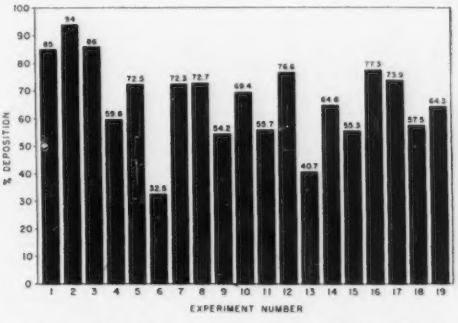

FIGURE 2. Percentage sodium chloride deposition in the respiratory tract in nineteen experiments on nine dogs.

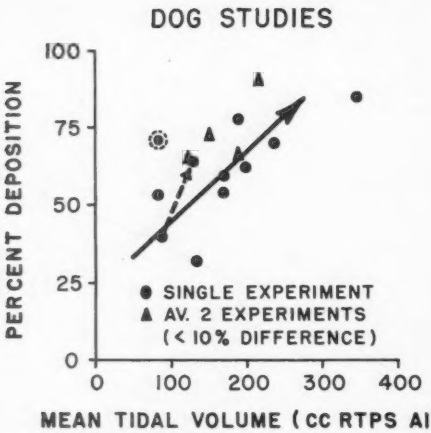

FIGURE 3. The heavy line (arrow) portrays the general trend of the data from all experiments. The dotted line connects two successive measurements on the same dog. The point encircled with dots is the value obtained from the experiment in which the dog was apparently overdosed with chlorpromazine (see Methods and Materials).

These results are remarkably similar to those obtained in the human study with respect to both the means and ranges (*loc cit*).

Because certain physiologic parameters, viz., tidal volume, respiratory frequency and the respiratory air flows, have appeared to be relevant to aerosol deposition in man during controlled breathing,[9, 10, 11, 12] these parameters were of particular interest in the dog study.

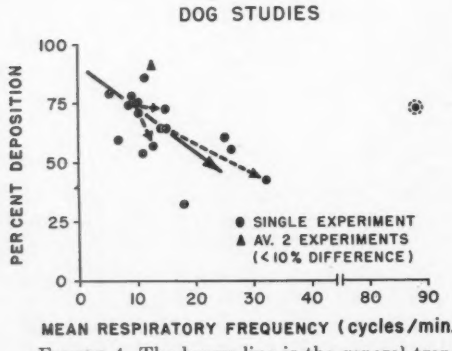

FIGURE 4. The heavy line is the general trend of the data. The dotted lines connect successive measurements on a dog whose respiratory frequency changed significantly. The point encircled with dots is from the overdosed dog. Note the pronounced tachypnea.

In Figure 3, the relation between increasing percentage deposition and increasing mean tidal volume appears to be significant. Not only does the relation generally hold among different experiments but in the instances when successive measurements were made, the relation appeared consistent. (If the mean values under comparison from two successive deposition measurements were within ±10%, they were not considered to be significantly different and were averaged together.) In Figure 4, the respiratory frequency is graphically related to the percentage deposition. Deposition increased with decreasing respiratory frequency. As in the case of the tidal volume data, the respiratory frequency bears this relationship in successive experiments and among different experiments. These relationships demonstrated in dogs (Figures 3 and 4) are consistent with those reported for man (*loc cit*).

The percentage deposition is plotted as a function of the mean inspired and mean expired air flow values in Figures 5 and 6, respectively. Though there appears to be a consistent relationship between an increasing inspired air flow and an increasing deposition, there is no obvious relation between the expired air flows and deposition.

The inspired air flow relationship reported in humans is qualitatively identical to that demonstrated in Figure 5. However, in the human studies, a relationship between the expired air flow and deposition was also suggested. It is

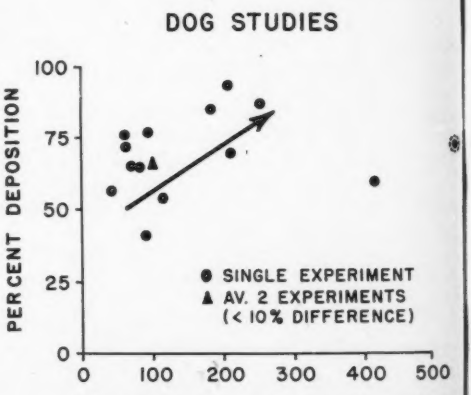

FIGURE 5. The heavy line is the general trend of the data. The dotted lines connect successive measurements on a dog whose inspiratory air flows changed significantly. Two points seem aberrant to the general trend line; one (encircled with dots) was obtained from the dog with the drug induced tachypnea.

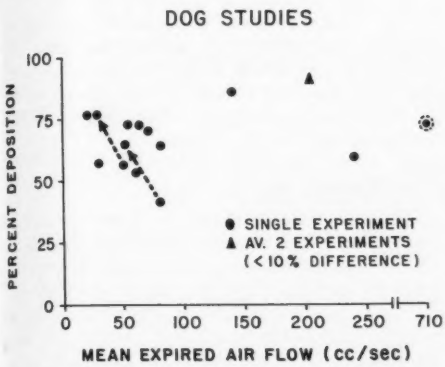

FIGURE 6. No general trend is apparent among the data. In two instances on successive measurements on the same dogs, (dashed lines) there was an increase in deposition with a decrease in the mean expired air flow. The point encircled with dots is from the dog with the drug induced tachypnea.

entirely possible that this was the result of some interdependence between parameters and that the expiratory air flow *per se* is not an important determinant. Moreover, the question of interdependency among these several physiologic parameters is appropriate to each and all of the parameters.

Discussion

While there have been several studies in man designed to study the effects of various physiologic factors on deposition, this is the first attempt at such an investigation in another animal species. The similarities in the apparent roles of certain physiologic parameters on deposition in man and dog are remarkable.

These similarities are particularly interesting since they demonstrate that even in uncontrolled breathing and with a heterogeneous aerosol, some physiologic factors are so intimately concerned with the deposition process that they can be revealed.

Possibly the most significant implication of the study is that under the proper experimental conditions, the dog is a suitable experimental subject for deposition studies whenever human subjects cannot be included.

Nevertheless, there are several factors which may cause the dog to respond differently. First, the nasal pharynx and trachea of the dog are disproportionately larger than man; this might produce a greater upper respiratory tract deposition, for example. Second, in certain situations the respiratory behavior of the dog has virtually

TABLE I

	Man	Dog
Lung Weight (gm/100 gm body wt.)	1.0 (0.7–1.5)	0.94
Tidal Volume (ml)	504 (285–895)	176 (90–434)
Tidal Volume (ml/kg body wt.)	8 (4–16)	10 (6–23)
Minute Volume (liters)	6 (4–11)	3 (2–8)
Respiratory Frequency (cy/min)	12 (6–20)	18 (8–38)
Effect of increased tidal volume on aerosol deposition	↑	↑
Effect of increased respiratory frequency on aerosol deposition	↓	↓
Effect of increased mean inspiratory air flow on aerosol deposition	↑	↑
Over-all percentage deposition for 0.04μ CMD sodium chloride aerosol	63.4 (52–79)	66.5 (32–94)

* Average values used for comparison were obtained from this study (*loc cit*) and from References 13, 14 and 15.

no parallel in man; specifically, during exercise or periods of elevated temperature, the dog develops a tachypnea or panting. Third, the lobes of the lungs of man and dog differ in organi-

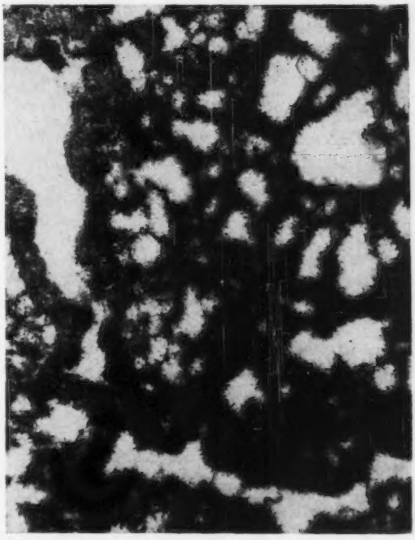

FIGURE 7. Autoradiogram of dog lung thirty days after inhalation of a Po^{210} tagged sodium chloride aerosol. Bronchiole at bottom edge of picture is approximately 100 microns diameter. Film exposed for period equivalent to 15 per cent radioactive decay.

zation and probably differ in regional ventilation and dust deposition. Fourth, if a question of dust retention and pulmonary injury is included in the scope of the study, then there is the obvious proposition that the tissue response of the dog lung to dust (chemical or radioactive) might differ from man and that the dust clearance mechanisms may also differ in important ways. Despite these potentially important differences, a considerable number of similarities have been established between man and dog with respect to their respiratory anatomy and physiology and to the deposition of dusts in their respiratory tracts. Some of these are summarized in Table I.

Based on the distribution, excretion and lung recovery of the alpha emitter, polonium^{210}, which was incorporated in many of these dog experiments, it is possible to estimate the amount of the aerosol which was deposited in the upper and lower respiratory tract. The details of the estimates are simple and orthodox but too lengthy to delineate here. In essence, one ascribes all of the highly mobile and rapidly excreted polonium (fecal) to that polonium deposited initially in the upper respiratory tract; the polonium recovered from the lung at sacrifice is considered to be the minimum lower respiratory tract value; and finally, the amount of polonium involved in systemic distribution and urinary excretion is analyzed in terms of existing data from oral and intravenous studies. By this approach, the intital lower respiratory tract deposition appears to have been equal to or greater than the upper respiratory tract deposition in all cases. Finally, in all autoradiograms of the dog lungs (30–150 days post-exposure), the parenchymal areas are filled with alpha tracks, Figure 7.

Summary

The method and apparatus used in a study of aerosol deposition in the respiratory tract of dogs is briefly described.

A mean percentage deposition value of 66.5 (σ 14.9) was determined in nineteen experiments on nine dogs while spontaneously breathing a submicronic sodium chloride aerosol (0.04 μ CMD σ_g 2.3).

Several physiologic parameters appear to be related to aerosol deposition in the dog. An increase in deposition was associated with an increase both in the mean tidal volume and in the mean inspiratory air flow and with a decrease in the mean respiratory frequency.

This study suggests that under the proper experimental conditions, aerosol deposition in the dog is comparable to that occurring in man; therefore, the dog should be considered to be especially appropriate for investigations in the field of inhalation toxicology.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Mr. K. Lauterbach and Mrs. Charles Spiegl in electron microscopy, Messrs. B. Clemons and R. Cobb in animal care, Mr. L. Casarett in autoradiography and Miss Harriet Yarrison and Messrs. H. S. Campbell and DeWitt Wood in radiochemical analysis.

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Progression of Experimental Silicosis in the Rat*

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A DISTINCTION must be made between activity and progression of a silicotic process. For the purpose of histopathologic evaluation the presence of granulation tissue or immature connective tissue in silicotic lung parenchyma is indicative of activity. Progression of silicosis implies that after exposure to silica dust has ceased there is a continuation or a resumption of expansive growth of old, collagenized or quiescent lesions or the initiation of new lesions, or both.

The features of progressiveness in silicosis have not been characterized and it is the purpose of this communication to describe them. Because of absolute control of exposure conditions and of timing, experimental animal lesions lend themselves better than human material to a study of these features. For these reasons it is the former that are the subject of this report.

Methods

Silicosis was produced in rats by one of three methods.

1. Rats were exposed to quartz dust in an inhalation chamber 30 hours weekly for 15 months and then held without further exposure for six to nine months. The dust cloud was generated by the atomization of an aqueous suspension of the dust at 90 psi, dried by passing through a heated tube, and subsequently bubbled through an impinger to remove the majority of the particles and aggregates greater than one micron. The average concentration of the dust was 28 mg/m³ of which 58% of the particles by number were one micron and less. The geometric mean was 0.47 μ by optical sizing (×970).

2. Another series of rats received an intratracheal injection of either 75 mg or 40 mg of quartz suspended in one ml of water. The material for injection was obtained by elutriation in 95% alcohol and had an average particle size of 0.8 μ by optical sizing (×970).

3. A third series of rats received an intratracheal injection of 25 mg of flint suspended in one ml of water. The sample injected had been atomized at 40 psi similarly to the inhalation experiment, and collected by electrostatic precipitation. The average particle size, determined from electron photomicrographs (×4600), was 0.2 micron.

The lungs of all rats were fixed by the intratracheal injection of formalin under a head of 10 cm water pressure. Sections were cut at seven micra and stained with hematoxylin-eosin or van Gieson's stain or both.

The method of relating the silica to histologic structures has been previously described.[1] Briefly, suitable fields were accurately located with a "Field-finder" (sold by W. & L. E. Gurley, Engineering Instruments, Troy, N. Y.) and photographed at a magnification of ×82. The coverslips were then removed and the slides were incinerated at 550°C for one hour. After cooling to room temperature the slides were immersed in concentrated hydrochloric acid for 30 minutes, washed in distilled water and air-dried. The same fields were then rephotographed at the same magnification, but under dark field conditions. A composite negative was prepared by superimposing the dark-field negative upon the corresponding bright field negative and carefully matching the landmarks.

Results

A number of fields were examined and photographed from each of the 23 rats used in this study. The following observations are illustrative of the main features that characterize progressiveness in silicosis:

1. On the periphery of some relatively cell-poor, heavily collagenized nodules, conical projections of immature, cellular connective tissue were found. This immature connective tissue generally contained more silica than could demonstrated in near-by, more mature portions of the nodules. (Figures 1 and 2.)

2. In the lung sections of animals that had received but a single intratracheal injection of silica dust and had been killed one year later,

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it was common to find small immature nodules in the same field largely occupied by mature, well-collagenized silicotic nodules. It was also more common to find a greater silica content within the small, immature nodules than was found in the well-collagenized ones. (Figures 3 and 4.)

3. Focal cellular and collagenous thickening of alveolar walls occurred with a considerably lower frequency than the two preceding observations and usually involved alveoli situated between large confluent nodules. Although the thickened alveolar walls rarely contained demonstrable silica, large amounts of silica were frequently present in the corresponding air spaces. (Figures 5 and 6.)

FIGURE 1. R-2455 injected intratracheally with 25 mg of flint dust and killed 22 months later. At the margin of a well-collagenized nodule, relatively poor in cells, is a conical extension of immature connective tissue poor in collagen and rich in cells. Van Gieson ×164.

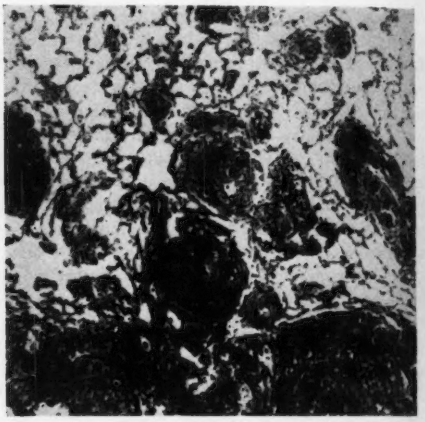

FIGURE 3. R-2539 injected intratracheally with 25 mg of flint dust and killed 13 months later. In contrast to the well-collagenized nodules in the lower half of the field, the nodules in the upper half of the field contain little or no collagen. This is particularly true of the small nodules. Van Gieson ×164.

FIGURE 2. Composite of the above showing a relatively abundant silica content of immature connective tissue at the margin of the nodule in contrast to the paucity of silica elsewhere. Van Gieson ×164.

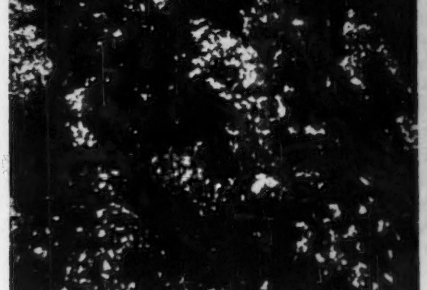

FIGURE 4. Composite of the above shows a much more abundant silica content of the immature nodules as compared with the more mature nodules. Van Gieson ×164.

4. The least frequent finding concerned small intra-alveolar nodules composed mainly of macrophages and their debris, but also containing a prominent reticulin network and few collagen fibers. The latter two clearly indicated the presence of cellular activity. Incineration studies demonstrated the presence of silica in such organizing foci thus justifying their designation as silicotic nodules. These immature intra-alveolar nodules were usually associated with near-by larger, mature nodules. (Figures 7 and 8.)

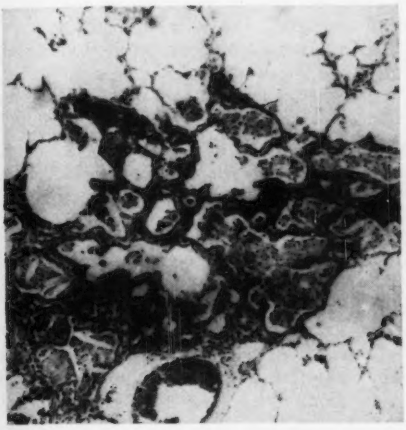

FIGURE 5. R-1319 exposed to quartz dust in an inhalation chamber for 15 months and then pastured for nearly 9 additional months before sacrifice. There is irregular thickening of cellular, noncollagenous character involving the walls of air spaces. The air spaces contain macrophages and debris. H. & E. ×164.

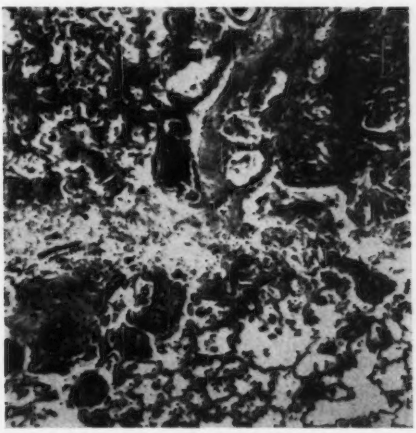

FIGURE 7. R-1339 exposed to quartz dust in an inhalation chamber for 15 months and died 6 months after removal from the chamber. In the lower left quadrant there are 3 intra-alveolar masses which contain only a small amount of collagen. Near-by air spaces contain variable content of macrophages and debris. H. & E. ×164.

FIGURE 6. Composite of the above showing a moderate amount of silica associated with the alveolar macrophages and debris. No significant silica content of the thickened walls is demonstrable. H. & E. ×164.

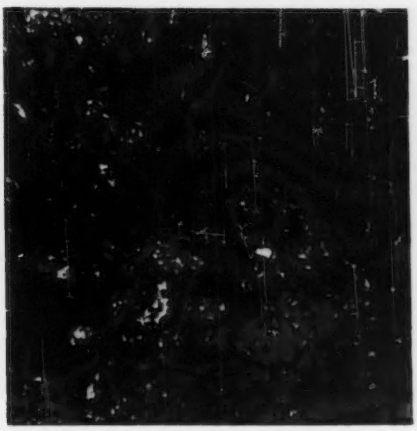

FIGURE 8. Composite of the above showing very little silica associated with the well-collagenized tissue and a more abundant amount of silica in intra-alveolar nodules as well as within the alveolar cells and debris. H. & E. ×164.

Discussion

Inasmuch as the lapse of one year's time following the last exposure to silica dust or after the single intratracheal injection of this material was apparently sufficient to allow for the near-maturation of most silicotic nodules, the occurrence of immature connective tissue associated with silica aggregates at the margin of otherwise quiescent nodules may properly be interpreted as representing activity of a silicotic process of relatively recent origin. A similar zone of immature connective tissue situated at the margins of human silicotic nodules had been designated by Simpson and Strachan[2] as the peripheral reactive zone.

Such activity of recent origin situated upon the periphery of an old, quiescent nodule should logically be viewed as a resumption of expansive growth. Similarly, the occurrence of immature nodules adjacent to old, mature nodules should be considered indicative of a recent initiation of new lesions. In the animals studied, the presence of cellularly thickened alveolar walls between old silicotic nodules and the organization of silicotic alveolar debris to form intra-alveolar nodules among old lesions are also evidence of the initiation of new lesions long after exposure to silica has ceased.

Summary

A distinction is made between activity and progression of silicosis. Progression of silicosis is defined as the continuation or a resumption of expansive growth of old, collagenized or quiescent lesions or the initiation of new lesions, or both after the exposure to silica dust has ceased.

The features of progressiveness in silicosis are described and consist of the following:

1. The occurrence of immature connective tissue at the margin of otherwise mature, quiescent nodules.
2. The occurrence of immature nodules adjacent to old, mature nodules.
3. The occurrence of cellularly thickened alveolar walls between old silicotic nodules in the animals studied.
4. The organization of alveolar debris to form intra-alveolar nodules.

Acknowledgments

Acknowledgment is given to Miss Ethel B. Tolker for her meticulous care in the preparation of the slides.

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THE MICHIGAN Industrial Hygiene Society Award for the Outstanding Paper in the 1957 volume of the American Industrial Hygiene Association Quarterly, was given to Paul W. McDaniel, Homer Fay, and Paul H. Mohr for their article, "Nitrogen Dioxide and Ozone Concentrations in Welding Operations." Mr. McDaniel (right) accepts the certificate and check for the authors from Warren A. Cook, President of the Michigan Industrial Hygiene Society. This was the first presentation of this annual award.

Toxicity Studies of Certain Organic Peroxides and Hydroperoxides*

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ORGANIC peroxides are likely constituents of certain types of oxidizing smog[1, 2, 3, 4, 5] and important as industrial catalysts, intermediates, and raw materials for synthesis.[6] Many of them are abundant sources of free radicals. For these reasons tests have been conducted in our laboratory to determine the toxicity of these compounds on small mammals. Specific compounds were selected on the basis of their known use in industry, as type constituents of oxidizing smog, their availability, and the feasibility of their use for extensive tests.

Materials and Methods

Physical and Chemical Properties of Peroxides

Some of the characteristics of the organic peroxides used, viz. di-t-butyl peroxide, t-butyl hydroperoxide, cumene hydroperoxide, and methyl ethyl ketone peroxide, are given in Table I: their specifications are given in Table II. Most of these values were furnished by the manufacturers.

The compounds were used as received from the manufacturer except when dilution was necessary, in which event propylene glycol, or in the case of methyl ethyl ketone peroxide, dimethyl phthalate was used.

Polarographic Method of Measurement

Because the conventional chemical methods (iodide or stannous chloride) are not specific for organic peroxides, whereas the polarographic method distinguishes between peroxides and hydroperoxides and is specific for determining both,[7] this method was selected as the most desirable for this study in which mixtures of compounds were involved.

Polarographic measurements were made with a Sargent Model XXI Visible-Recording Polarograph, using a dropping mercury electrode assembly and an improvised saturated calomel electrode which made contact with the test solution by way of an agar-KCl bridge. This agar bridge was suspended over the test solution (1:1 benzene:methanol) during the de-aeration period, then lowered into the solution before recording the polarogram. The polarographic method was that of Willits, et al.,[8] although the electrolysis vessel was of one author's (E.P.F.) design, patterned somewhat after that of Bruschweiler and Minkoff.[9] According to Willits, et al.[8] both the hydroperoxide and peroxide half-wave potentials are widely separated and the polarographic method not only identifies the functional groups but also gives a quantitative measure of each in peroxide-hydroperoxide mixtures. Di-t-butyl peroxide is an exception to this in that it is not reduced polarographically.

Animal Species and Techniques

The rats used in these experiments were Wistar-derived adult, male, albinos weighing approximately 200 gm at the beginning of each test. The mice were Swiss-derived adult, male, albinos having an initial average weight of 20 gm each. The rabbits were white males of the New Zealand strain weighing from 2.7–6.5 kg.

Standard laboratory pellets and drinking water were available to all animals at all times except during exposure or fasting periods. The rats were housed five to a cage, the mice, ten to a cage, and the rabbits, one to a cage. The rats and mice were sacrificed by cervical dislocation; the rabbits, by overdosage of Nembutal.

Acute studies were performed by administering organic peroxides by the intraperitoneal and oral routes on rats, and by the inhalation route on both rats and mice. The calculated LD_{50} values for the intraperitoneal and oral routes were determined according to Weil[10, 11] in which five rats were used for each of four geometrically spaced dosage levels. Three of the organic peroxides were diluted (volume/volume) as follows to accomplish easier intraperitoneal administration: 20% t-butyl hydroperoxide in propylene glycol, 10% cumene hydroperoxide in propylene glycol, and 5% methyl ethyl ketone peroxide in dimethyl

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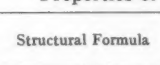
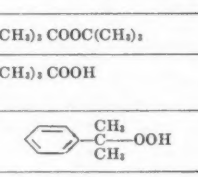
TABLE I
Properties of Organic Peroxides Used

Compound	Structural Formula	M.W.	Sp.G.	M.P. C°	B.P. C°(mm Hg)	$E_{\frac{1}{2}}$* Volts
Di-t-Butyl Peroxide	$(CH_3)_3\,COOC(CH_3)_3$	146.22	0.794 20/4	−40		None
t-Butyl Hydroperoxide	$(CH_3)_3\,COOH$	90.12	0.896 20/4	−8 to −10	111 (760) 80 (284)	−1.09
Cumene Hydroperoxide		152.18	1.048 15.6/15.6		65 (.18)	−0.92
Methyl Ethyl Ketone Peroxide[19]		ca. 185.	1.12 15/15		d. 118	−1.08

* Polarographic half-wave potentials were corrected for IR drop, but were determined on the compounds as received from the manufacturer without further purification. Table II indicates the purity of these compounds. Willits, et al.[8] report the $E_{\frac{1}{2}}$ of *purified* t-butyl hydroperoxide and cumene hydroperoxide as −0.96 and −0.68 volt respectively.

phthalate. In all other cases the peroxides were used as received from the manufacturer without further dilution. The rats used for oral tests were fasted 24 hours previous to treatment. Six rats and ten mice were used for each exposure level for the inhalation studies. These exposure levels were determined by measuring the liquid volume of the compound used for the duration of the exposure (15 liters of air/minute for four hours) and computing the concentration. Polarographic analysis of occasional samples (10 liters per minute for 5 minutes each) of the chamber atmosphere for organic peroxide gave concentration values ranging from 91% to 102% of these calculated values, with an average of 97%. Air from the laboratory compressed-air line was cleaned and dried before passing into the exposure chamber. Some of this air was passed through an aerosol unit (containing the liquid organic peroxide) at a measured rate of flow. This inhalation-exposure apparatus was constructed of glass and patterned after one used by Svirbely and Saltzman.[12]

TABLE II
Specifications of Organic Peroxides Used

Compound	Manufacturer*	Per Cent Purity (Minimum)	Per Cent Active Oxygen (Minimum)	Other Components
Di-t-Butyl Peroxide	Lucidol	97	10.6	Fe (trace)
t-Butyl Hydroperoxide	Lucidol	70	12.5	Di-t-B.P. (20%)
Cumene Hydroperoxide	Hercules	73	7.7	H_2O (0.4%), Parent Hydrocarbons, and derivatives
Methyl Ethyl Ketone Peroxide	Cadet & Lucidol	60	11.0	Dimethyl phthalate (40%)

* Lucidol: Division of Wallace & Tiernan, Inc., 1740 Military Road, Buffalo 5, New York; Hercules: Hercules Powder Co., Wilmington 99, Delaware; Cadet: Cadet Chemical Corporation, Burt, New York.

Subchronic tests, to study the possible cumulative effects and the resultant histopathology were performed on rats by repeated intraperitoneal and oral doses one-fifth that of the LD_{50} three times weekly (Monday, Wednesday, Friday) for seven weeks.

A histopathologic study involving serial sacrifices was made on rats injected intraperitoneally in an attempt to find some consistent pathologic change due to an organic peroxide. The rats were given sublethal doses of organic peroxides in three injections during one week (Monday, Wednesday, Friday) and one-third of the group sacrificed at 1, 2 and 3 weeks after the first injection. An unexposed control group of rats of the same age was sacrificed with the exposed group.

For testing the toxicity of organic peroxides on the rabbit eye, the technique of Draize and Kelly[13] was used, by which the reactions observed in the cornea, iris, and the palpebral and

bulbar conjunctivae were scored separately. This method of assigning numerical scores gives as much as 80% of the weight to the observed lesions in the cornea and iris, the structures of the eye most concerned with vision. The ocular reactions were read with the aid of a hand slit-lamp.

Skin tests for primary irritation were performed on a similar group of rabbits by the standard technique used in this laboratory by which a large area of the back of each rabbit was sheared closely, and one or two drops of each compound applied at a different spot on this sheared area (as many as six spots per rabbit), and spread to a circular area about two centimeters in diameter. At each reading the rabbit was sheared and the area wiped clean with a wet cloth for subsequent readings which were made at 24, 48 and 72 hours respectively. The severity of the reaction was graded as follows: 0 for negative, 1 for erythema, 2 for erythema and edema, 3 for erythema, edema and slight vesiculation, 4 for erythema, edema and severe vesiculation or bullous formation.

To test for possible changes in serum protein, rabbits were treated as in the above tests for primary irritation, using one rabbit per compound. Electrophoretic patterns were made on the serum from 3–5 ml of blood taken from the rabbit's ear before and after exposure. The Spinco Paper Electrophoresis Cell, Model R-Series D, with the Spinco Duostat Regulated D. C. Power Supply, and the Spinco Analytrol Recording Scanner and Integrator for translating the dye densities into "ticks" were used to test for changes in serum protein. The technique was that of Durrum.[14]

Tests for methemoglobin according to the technique of Evelyn and Malloy[15] were made on blood secured from the rat heart by the method of Burhoe.[16] The Beckman Ratio-Recording Spectrophotometer Model DK-2 was used to record the per cent light transmission from 350 to 700 $m\mu$ wave length.

Results of Acute Tests

Intraperitoneal Injections, Rats

Methyl ethyl ketone peroxide, t-butyl hydroperoxide and cumene hydroperoxide were more toxic by all routes examined by a factor of from 40–150-fold than di-t-butyl peroxide as shown by the LD_{50} values given in Table III. All of the rats showed signs of weakness and some loss of equilibrium following single doses of all but the lowest levels of each of the above four organic peroxides. Prostration occurred frequently at the higher dose levels and was usually followed by death, although some of the rats recovered completely. This was particularly conspicuous in the animals treated with methyl ethyl ketone peroxide in which there was one survivor of five rats treated with 80 mg/kg and five survivors of five treated with 40 mg/kg. Prostration had occurred in all. Porphyrin deposition in the nostrils occurred in many of the rats following a single injection of cumene hydroperoxide (200 mg/kg) and di-t-butyl peroxide (2000 to 5000 mg/kg). The pelage was coarse in most of the rats at two to five days following a single dose (excepting the lowest) of each of the four organic peroxides. Shivering occurred in rats following high doses of di-t-butyl peroxide. Practically all of the deaths from intraperitoneal injection of organic peroxides in these acute studies occurred within six days. There was no weight loss in any of the surviving animals during the four-week period following a single injection at each dose level, however.

Oral Administration, Rats

The LD_{50} value for di-t-butyl peroxide by gavage was greater than 25,000 mg/kg; amounts greater than this distended the rat's stomach beyond the point where the results would have toxicologic significance.

The three hydroperoxides were found to have LD_{50} values of the same order of magnitude ranging from 382 mg/kg for cumene hydroperoxide to 484 mg/kg for methyl ethyl ketone peroxide (Table III).

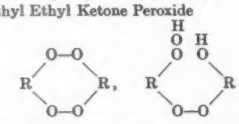
Practically all of the deaths from the oral administration of organic peroxides occurred within five days. There was no weight loss in any of the surviving rats during the four-week period following a single dose administration of each dose level. Extensive urinary bleeding occurred in the rats treated with cumene hydroperoxide (400 mg/kg).

Inhalation Exposure, Rats and Mice.

The LC_{50} values (Table III) were comparable for rats and mice, the greatest divergence again occurring in the case of di-t-butyl peroxide; 4103 ppm, was the highest attainable vapor concentration under the conditions of the experiment and neither rats nor mice succumbed from a 4-hour exposure at this level. Tertiary-butyl hydroperoxide by inhalation tests is seen to be less toxic also than either methyl ethyl ketone peroxide or cumene hydroperoxide, whereas they were found to be of approximately equal toxicity by the intraperitoneal route (65 and 95 mg/kg) or by oral administration (484 and 382 mg/kg).

TABLE III
Toxicity Limits of Four Organic Peroxides

Compound	LD_{50}		LC_{50}		Maximal Non-irritating Strength	
	mg Compound/kg Body Weight		ppm 4 hours exposure		Per Cent Peroxide in Vehicle	
	Intra-peritoneal	Oral (Gavage)	Inhalation		Skin	Eye
	Rats	Rats	Rats	Mice	Rabbits	Rabbits
Di-t-Butyl Peroxide (ROOR)	3210	>25000	>4103	>4103	97	97
t-Butyl Hydroperoxide (ROOH)	87	406	500	350	35	7
Cumene Hydroperoxide (ROOH)	95	382	220	200	7	1
Methyl Ethyl Ketone Peroxide	65	484	200	170	1.5	0.6



Rats exposed to di-t-butyl peroxide vapor (4103 ppm) developed head and neck tremors uniformly in all six rats after ten minutes of exposure. A weekness in the extremities occurred, appearing first in the forelegs, later in the hindlegs. After 45 minutes the tremors lessened in all the rats. After 1 hour and 45 minutes one of the rats showed signs of hyperactivity at 10-second intervals. At the end of the 4-hour exposure the tremors had virtually disappeared, and the rats remained prostrate. Seven days later the hyperactive rat died; the only death of the six exposed rats.

The mice were less affected than the rats, exhibiting only excitability and somewhat labored breathing. This occurred after ten minutes of exposure, was maximal at 30 to 50 minutes, and still persisted, though somewhat lessened, at the end of the exposure period. The high dosage levels of each of the peroxides tested by the inhalation route caused porphyrin deposition in the nostrils and irregular respiration in the majority of the rats and mice.

Skin Tests, Rabbits

There was apparently no immediate discomfort caused by the direct cutaneous application of the organic peroxides; the delayed reaction was quite severe, however, for three of the compounds, t-butyl hydroperoxide, methyl ethyl ketone peroxide, and cumene hydroperoxide. When applied full strength these three compounds caused erythema, edema and vesiculation within two or three days. Methyl ethyl ketone peroxide again exhibited the greatest toxicity of the four organic peroxides tested, di-t-butyl peroxide the least. The maximal nonirritating strengths of the peroxides tested are given in Table III.

Eye Tests, Rabbits

The maximal nonirritating strengths of these peroxides are given in Table III. The total scores expressing the effects of various concentrations of organic peroxides on the eye mucosa are given in Table IV. With the exception of di-t-butyl peroxide, strong solutions of organic peroxides in dimethyl phthalate or propylene glycol when applied directly to the eyes of rabbits affected the cornea, iris, and conjunctiva extensively. Weaker solutions (Table IV) affected

TABLE IV
Toxicity of Organic Peroxides to Rabbit Eye Mucosa*

Compound	Strength %	Day of Reading Following Treatment					
		1	2	3	4	6	7
Di-t-Butyl Peroxide	97	0	0	0	0		0
t-Butyl Hydroperoxide	35†	59	57	79	75		46
t-Butyl Hydroperoxide	7†	4	13	4		0	0
Cumene Hydroperoxide	10†	59	59	77	75		44
Cumene Hydroperoxide	1†	11	6	4		0	0
Methyl Ethyl Ketone Peroxide	3‡	57	57	11	9		7
Methyl Ethyl Ketone Peroxide	0.6‡	0	4	0		0	0
Control	0	0	0	0		0	0

* Ocular lesion score of Draize & Kelly.[6]
† Dilutions made with propylene glycol.
‡ Dilutions made with dimethyl phthalate.

only the conjunctiva causing redness of the palpebral conjunctiva and chemosis. Methyl ethyl ketone peroxide, which is a mixture of dimeric peroxide and hydroperoxide, was found to be particularly marked as an eye irritant. Washing the rabbits' eyes with water four seconds after application of the peroxides prevented reactions in every case.

Results of Repeated Administration

Intraperitoneal Injections, Rats

With dosages of ⅕ the intraperitoneal LD_{50} thrice-weekly for 7 weeks, cumene hydroperoxide and t-butyl hydroperoxide, each produced death in one of five rats; methyl ethyl ketone peroxide killed two of five; whereas di-t-butyl peroxide killed none during the 7-week period, as shown in Table V. All of the rats gained weight normally throughout the test period, although the pelage of some was noticeably coarse.

Oral Administration, Rats

Table V shows that methyl ethyl ketone peroxide produced death in five of five rats treated under the same dosage schedule described for repeated intraperitoneal injections, cumene hydroperoxide killed four of five, di-t-butyl peroxide, two of five, and t-butyl hydroperoxide, none during the 7-week test period. Loss of weight, or the failure to gain normally, was a conspicuous feature of this test. The pelage was coarse in most of the rats after the first week of treatment.

Pathology

Acute Tests

Autopsy of several of the mice and rats killed by the exposure to organic peroxide vapor or sacrificed within the first 24 hours following exposure showed hyperemia of the lungs, with petechial hemorrhages on the lung surface of some and gross hemorrhage in others. No other lesion found in the acute studies (by inhalation, oral, and intraperitoneal routes) by either gross or histologic examination could be attributed to organic peroxides. However, there was occasional damage to the liver (fatty changes in liver cells in central portion of lobules, increase in number of round cells in portal spaces, or mild hyperemia), and to the kidney (granular precipitate or casts in the lumina of the convoluted or collecting tubules, and desquamation of the epithelium of the proximal tubules).

Table V
Mortality of Rats from Repeated Doses of Organic Peroxides
Five Animals Used per Test.
Dosage: ⅕ LD_{50} of each route, three times weekly on alternate days.

Organic Peroxide	Cumulative Mortality by Weeks													
	Intraperitoneal							Oral (Gavage)						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Di-t-Butyl Peroxide	0	0	0	0	0	0	0	0	1	2	2	2	2	2
t-Butyl Hydroperoxide	0	0	1	1	1	1	1	0	0	0	0	0	0	0
Cumene Hydroperoxide	0	1	1	1	1	1	1	0	1	2	3	4	4	4
Methyl Ethyl Ketone Peroxide	1	1	1	1	1	2	2	1	2	3	3	4	5	5

Serial Sacrifice Study

In the rats injected with t-butyl hydroperoxide there was some depletion of glycogen in the liver and the liver cells were slightly more homogeneous than in the controls, but no structural changes were observed. A number of rats had chronic interstitial pneumonia, apparently unrelated to the exposure.

In the rats injected with methyl ethyl ketone peroxide the liver was mildly damaged in all. All rats at first week sacrifice showed depletion of liver glycogen and dissociation of liver cords. At two and three weeks there was still low glycogen content but no dissociation of liver cells. The control rats showed none of these changes, but these changes are frequently found in the liver due to agonal stress. Chronic interstitial pneumonia and bronchiectasis were seen in a large percentage of rats and were consistent with the findings in spontaneous murine pneumonia.

Results of the Special Tests

Cross-Tolerance

Experiments were performed to determine whether cumene hydroperoxide would afford protection from subsequent lethal concentrations of itself, hydrogen peroxide, ozone and nitrogen dioxide separately. For this, a group of 45 mice was exposed to an atmosphere of 100 ppm cumene hydroperoxide for six hours. (The LC_{50} by inhalation is 220 ppm for a 4-hour exposure). The mice in groups of ten were challenged one week later to the vapors of either cumene hydroperoxide, hydrogen peroxide, ozone and nitrogen dioxide at the 4-hour lethal concentration or above it. As a control, a group of ten mice of the same age and weight not pre-

TABLE VI
Comparison of Albumin/Globulin Ratios in the Serum of Rabbits Treated Cutaneously with Organic Peroxides

	Day of Reading Following First Application							
	0*	1	2*	5*	7	9	12	14
Di-t-Butyl Peroxide	1.6	1.4	1.2	1.7	1.8	2.0	1.4	2.0
t-Butyl Hydroperoxide	1.0	1.0	1.1	1.1	1.2	1.2	1.2	1.4
Cumene Hydroperoxide	1.3	1.5	1.3	1.6	1.2	1.2	1.5	1.4
Methyl Ethyl Ketone Peroxide	1.2	1.2	1.3	1.1	1.1	1.5	1.4	1.6
Propylene Glycol	1.3	1.7	1.2	1.3	1.2	1.4	1.2	2.0
Dimethyl Phthalate	1.4	1.5	1.6	1.4	1.3	1.5	1.5	1.6

* 0.1 ml of compound applied to a different area of the rabbit's back (after clipping) on each of these dates.

viously exposed to cumene hydroperoxide accompanied the pre-exposed mice when challenged by each of the four substances. Cumene hydroperoxide afforded protection only against hydrogen peroxide vapors. Challenging concentrations of hydrogen peroxide at 226 ppm for four hours (the approximate LC_{50} for mice) resulted in a mortality of 10% of the pretreated mice, against 50% mortality for the controls. No protection was afforded against the other respiratory irritants, including cumene hydroperoxide.

Methemoglobin Formation in Rats

It was found that methyl ethyl ketone peroxide formed methemoglobin in rat blood *in vitro*, but not *in vivo*. Repeated daily exposures of rats by inhalation at low levels for three days in succession, or repeated intraperitoneal injections three times a week for five weeks failed to develop any significant amount of methemoglobin.

Changes in Serum Proteins.

The albumin/globulin ratio increased appreciably over a 2-week period (three applications and eight readings as indicated in Table VI) for rabbits treated as in the tests for primary irritation with di-t-butyl peroxide, t-butyl hydroperoxide and methyl ethyl ketone peroxide. But it also increased similarly in rabbits treated with propylene glycol and dimethyl phthalate, and not at all for rabbits treated with cumene hydroperoxide.

Discussion

The toxicity values in Table III are expressed as pure compounds, although as seen in Table II, none was absolutely pure and three of them contained appreciable amounts of stabilizing vehicles. The toxicity limits of the four organic peroxides determined in these studies revealed marked differences. Particularly noteworthy was the finding that the hydroperoxide (ROOH) was far more toxic than its dimeric peroxide (ROOR), by all routes of administration. Toxicity studies reported elsewhere support this finding; dicumyl peroxide was found to have an oral LD_{50} (single dose for rats) of 3500-4000 mg/kg compared with 382 mg/kg for cumene hydroperoxide found by the present authors. This same report[17] indicated that dicumyl peroxide was relatively nonirritating to rabbit eyes, whereas the present authors found cumene hydroperoxide to be extremely irritating to rabbit eyes. Although the methyl ethyl ketone substance was considered a peroxide, it was actually a mixture of peroxide and hydroperoxide, as noted earlier; its toxicity bears this out. As a group, one would rate the organic peroxides, according to the classification of Hodge and Sterner[18] as "moderately toxic" (oral LD_{50}, single dose, rats) although one of them, di-t-butyl peroxide, would be classed as "relatively harmless". By the inhalation route the classification would be moderately toxic" and "slightly toxic" respectively.

One possible correlation, between the degree of toxicity and the properties of these compounds (Tables I and II) may be found in the polarographic half-wave potential, E½. Di-t-butyl peroxide, the least toxic of the group, was not reduced by the polarographic technique, whereas the other organic peroxides, t-butyl hydroperoxide, methyl ethyl ketone peroxide, and cumene hydroperoxide, which as a group are "moderately toxic" compounds, have comparable half-wave potentials (−0.92 to −1.09 volts).

The physiologic responses in the acute tests were mild except for the head and neck tremors described for rats and mice exposed to di-t-butyl peroxide vapor. The most common response was that of weakness and coarse pelage, which was particularly conspicuous following intraperitoneal injections. Nasal porphyrin exudate occurred occasionally in the acute intraperitoneal and inhalation tests. Most of the rat deaths in the acute studies occurred within 48 hours after the administration of organic peroxides, and practically all of the deaths occurred within 5 or 6 days. Oddly, gain in weight was normal in all of the acute tests, as well as during the 7-week injection tests, but not in the 7-week oral tests.

The skin and eye tests indicated that the hydroperoxides, cumene hydroperoxide, t-butyl

hydroperoxide, and methyl ethyl ketone peroxide were irritants, although their effects were not immediate: di-t-butyl peroxide was not irritating to either the skin or eye. None of the organic peroxides caused irritation when washed from the rabbits' eyes four seconds after application.

The fact that animals died from repeated administration of small doses (⅕ LD_{50}) of organic peroxides is interpreted as a cumulative effect, and probably of considerable importance because in most instances (Table V) cumulative deaths occurred by intraperitoneal as well as the oral route. No explanations are given to account for why di-t-butyl peroxide did not show cumulative toxicity intraperitoneally when it was rather marked orally; nor why the hydroperoxide showed no cumulative mortality orally. There was no conspicuous physiologic response to the repeated intraperitoneal injections except for the development of rather coarse pelage in some of the rats. The weight gain was normal throughout the test period. The more marked cumulative mortality resulting from the oral administrations would indicate lesser detoxication by this route. Associated with this was a conspicuous loss of weight and development of coarse pelage in all of these tests.

There was no pathologic change that could be attributed specifically to the organic peroxides. Changes in the liver were typical of those frequently found following agonal stress, and the lung changes were considered consistent with those found in spontaneous murine pneumonia. The lungs of some rats and mice were found by gross examination to be hyperemic and slightly hemorrhagic following exposure to organic vapors.

Of the special tests performed with the organic peroxides it was found that rats, following exposure to the vapor of cumene hydroperoxide developed an appreciable tolerance to the challenging lethal doses of the vapors of hydrogen peroxide, although oddly no tolerance to cumene hydroperoxide itself could be demonstrated. Another test showed that whereas methyl ethyl ketone peroxide produced methemoglobin in rat blood *in vitro*, repeated inhalation exposures and intraperitoneal injections of methyl ethyl ketone peroxide failed to produce methemoglobin in rat blood *in vivo*.

Summary

Of the four organic peroxides studied (methyl ethyl ketone peroxide, cumene hydroperoxide, t-butyl hydroperoxide, and di-t-butyl peroxide), methyl ethyl ketone peroxide was the most toxic by all five routes of administration. Toxicity ratings indicate that the hydroperoxides (ROOH) are "moderately toxic" compounds. Di-t-butyl peroxide (ROOR) was the least toxic by all routes tested and is classed as "relatively harmless" to "slightly toxic" (inhalation).

Histopathologic study failed to reveal any site of damage, although there were questionable indications that the liver may be involved.

Some protection from lethal doses of hydrogen peroxide was demonstrated in rats following exposure to cumene hydroperoxide and subsequent challenging with hydrogen peroxide vapors.

It was demonstrated in rats for at least three of the four organic peroxides tested that repeated sublethal doses (⅕ LD_{50}) either orally or intraperitoneally resulted in cumulative effects ending in death of some of the animals.

All of the organic peroxides tested were skin and eye irritants, except di-t-butyl peroxide.

Changes in the electrophoretic pattern of the serum protein of rabbits, or the presence of methemoglobin in rat blood could not be conclusively demonstrated in animals following exposure to organic peroxides.

Acknowledgments

We wish to acknowledge the following persons for assisting in this study: Dr. Richard Mendenhall for the electrophoresis work, Dr. Olga Dobrogorski for the histopathology study, Dr. J. L. Svirbely for the "cross-tolerance" experiment involving hydrogen peroxide, Vernon B. Perone for the rabbit skin and eye tests, and Dr. Jacob Berghuis for the eye mucosa readings.

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DR. CHARLES R. Williams, retiring President, (center) presents a certificate of Honorary Membership to Dr. A. J. Lanza on behalf of the American Industrial Hygiene Association, while incoming President, Kenneth W. Nelson (right) looks on.

Studies on Smog Produced in Irradiated Reaction Chambers*

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THE WORD "smog" is not very well defined as yet—most dictionaries still call it "a mixture of smoke and fog," and let it go at that. For this reason, in giving a talk on smog, it is a good idea to start out by defining our terms.

We think of smog at our laboratory in terms of two sets of properties characterizing each of two types of smog. These properties are outlined in Table I.

We consider that there are two types of smog. One of these occurs most typically in Los Angeles, and the other most typically in London. The differences between the two are obvious.

The work upon which we are reporting has been on the Los Angeles type of smog, and was performed under contract with the Air Pollution Foundation, which is an independent, non-governmental, non-profit organization formed to study the causes and manifestations of smog in Los Angeles. We are indebted to Dr. W. L. Faith and Dr. L. H. Rogers of the Air Pollution Foundation for their support during the course of this work. The definition of the two types of smog we have just given in Table I is due to Dr. Rogers.

Our assignment throughout the three-year duration of our project, from summer 1954 through summer 1957, was to use motor car exhaust and certain hydrocarbons typically found in it, oxides of nitrogen, and irradiation by actinic light to simulate Los Angeles smog in a suitable chamber in which it could be studied under controlled conditions.

Our initial studies, the object of which reflected the state of knowledge of Los Angeles smog in 1954, were attempts to produce smog damage in plants, specifically pinto beans, on the basis that this would be a completely objective sort of thing, devoid of the subjective aspects of evaluating eye irritation in human beings.

Previous investigators had reported that the most typical aspects of Los Angeles smog—odor, eye irritation, and plant damage—could be produced by mixing ozone and a suitable hydrocarbon together under the proper conditions. Of the hydrocarbons studied, 1-hexene appeared to be the most effective. Other investigators reported that motor car exhaust was a suitable source of the hydrocarbon component. These workers indicated that the hydrocarbon content of motor car exhaust was far more effective in producing smog than was 1-hexene on a comparable weight basis. If these results could be taken as valid—if the total hydrocarbon content of motor car exhaust is more effective in producing smog than is 1-hexene—then there must be one or more unknown components of the exhaust more effective than 1-hexene, since all known components are less effective. Our initial study was designed to attempt to isolate and identify these components.

Unfortunately, over a period of almost a year, it became more and more apparent from our own work and that of other investigators that there is no great degree of correlation between plant damage from smog and eye irritation from smog, and that attempting to assess plant damage and assign its causes to the presence of smog, ozone, or other phytotoxicants is by no means as objective a sort of thing as was expected. Measurement of degree and type of plant damage had to be assessed strictly by visual observation, and this assessment turned out to be about as subjective as direct measurements of eye irritation.

We, therefore, turned to eye irritation studies to measure smog effects, and by means of extensive replication of experiments and fairly sophisticated statistical treatment of the results, we were able to reach a number of significant conclusions, which will be discussed in the remainder of this paper.

About the same time that our program began to emphasize eye irritation as the primary measurement of smog intensity, the Air Pollution Foundation set up at the South Pasadena, California, facility of Stanford Research Institute a dynamic system for smog investigations, in which a constant flow of motor car exhaust is added to an air stream. This mixture is then irradiated by actinic light from artificial sources in a reac-

* Presented at the 1958 Industrial Health Conference, Atlantic City, N. J., April 24, 1958.

TABLE I

Los Angeles Type	Smog Characteristics	London Type
75–90°F	Temperature	32°F
50%	Relative Humidity	90%
Day (10 A.M.–2 P.M.)	Occurrence	Any time, day or night
Subsidence	Inversion Type	Radiation
Petroleum gases	Composition (initial)	Coal gases and particulate matter
¼ Mile	Visibility	None
Eye	Irritation	Mucous membranes
Photochemical (net oxidizing)	Reaction Type	Thermal (net reducing)

tion chamber of 500 cubic foot volume. Simultaneously, an equal flow of polluted air issues from the chamber and is subjected to various chemical and physiological tests.

This facility at Stanford Research Institute may be compared and contrasted with ours, which differs in a number of respects. Most important of these is that ours operates as a static, or batch system. A single parcel of polluted air is produced in the reaction chamber at a given time and, without further addition of reactants, tests are run on samples of this parcel of polluted air over a period of time. Further differences between our system and the one at Stanford Research Institute are that ours is over four times as large and it is irradiated with natural sunlight.

Our facility for studying air pollution, then, tends to simulate a limiting condition which might be found in an actual atmosphere where it is stagnant and receives no further pollution beyond the initial dose.

It was the primary purpose of the Air Pollution Foundation in supporting more or less similar programs at both these facilities—the dynamic one and our static one—to compare the results obtained in each and to allow each program to strengthen the other by simultaneous study of the smog problem from two different angles, and, finally, to use the results of both of these programs to help answer the basic question: How much and in what ways will motor car exhaust emissions need to be controlled in order to minimize present and future smog conditions?

Let us now pass on to a description of our smog facility at Midwest Research Institute. This was adapted from a small greenhouse, as shown in Figure 1. This figure also shows one of the motor cars used for exhaust production. This 1952 Ford station wagon with automatic transmission was later replaced by a similar 1956 Ford station wagon.

Both cars were kept in good condition by frequent tuneups, and operating characteristics of the engines were kept under control by means of such instruments as a tachometer, a manifold vacuum meter, and, most important for proper operation, a Liston-Becker non-dispersive infrared analyzer, sensitized to hexane as a typical hydrocarbon and connected to give a constant measurement of the hydrocarbon content of the exhaust gases.

The exhaust from the car is led into the smog facility by a short piece of flexible metal pipe carrying a double-throw spring-operated valve, so that precisely timed charges of exhaust can be introduced. The interior of our smog facility is shown in Figure 2, which shows the modifications necessary to convert the greenhouse into a suitable facility for smog studies. A glass floor was installed and glass panels were set up to cover the metal heating coils and concrete foundation wall. All other surfaces are coated with a white alkyd resin paint. It is calculated that 80% of the chamber surface is glass. The volume of each chamber is 2200 cubic feet, and

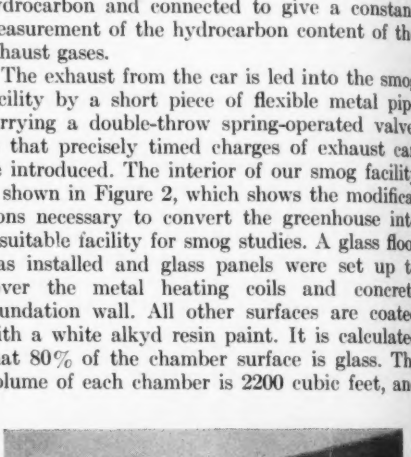

FIGURE 1. Exterior of Midwest Research Institute Smog Facility.

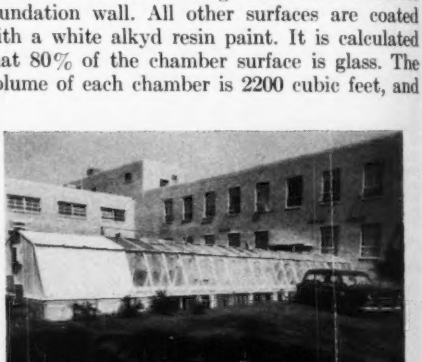

FIGURE 2. Interior of Smog Facility. Showing Eye Irritation Panel.

the volume to surface ratio is 2 cubic feet per square foot.

All joints are well caulked, and soft weather-stripping around the doors makes the chamber fairly air tight. Spring latches insure complete closing of the doors. Fans at the floor level promote rapid mixing of the pollutants introduced. Materials such as nitric oxide, nitrogen dioxide, and hydrocarbon in the vapor state, all under conditions where they would not be expected to undergo chemical reaction, have an approximate half-life of 90 minutes. This loss is probably mostly due to ventilation, but there is also a degree of surface adsorption to be expected.

Now let us describe our analytical instrumentation for this smog facility. You see in Figure 2 our most important analytical instrument—our smog panel. These people enter the chamber at specified times during an experiment, stay a specified period, leave the chamber, and report on a form the eye irritation they have experienced, on a scale of seven values ranging from none to very severe. Under normal conditions of a test, the panel members wear respirators to prevent nose and throat irritation and keep this effect from affecting their estimate of eye irritation.

Figure 3 shows our next most important instrument, which is a Kruger Oxidant Recorder. This continuously measures and records the extent to which air flowing through the instrument at a measured rate has the ability to oxidize iodide to tri-iodide in a buffered solution of potassium iodide. The importance of this instrument is due to the general belief that there is a correlation between eye irritation and oxidizing capability of the atmosphere. Our work, as will be seen, indicates strongly that there is no such correlation.

Also visible in this same figure is part of our collection system for air samples for each of the two chambers. By means of glass tubing leading to each chamber, vacuum pumps, and flowmeters, measured samples of chamber air can be taken for various wet chemical tests. Among those we have run from time to time are oxidant by phenolphthalin oxidation, oxidant by ferrous thiocyanate oxidation, aldehydes by reaction with bisulfite and subsequent iodine titration, hydrocarbons by infrared spectrophotometry of freeze-out samples, and sulfur dioxide by absorption in sodium tetrachloromercurate followed by color formation by treatment with acidic pararosaniline. From time to time oxidant has also been measured directly in the chambers by the rubber-cracking method using the Goodrich ozonometer, and carbon monoxide has been measured directly in the chambers by use of a Mine Safety Appliances carbon monoxide meter.

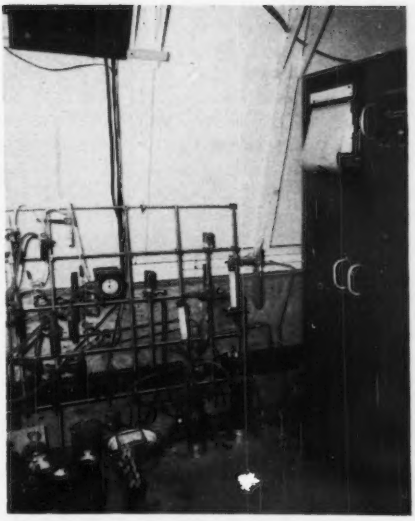

FIGURE 3. Oxidant Recorder and Air Sampling Assembly.

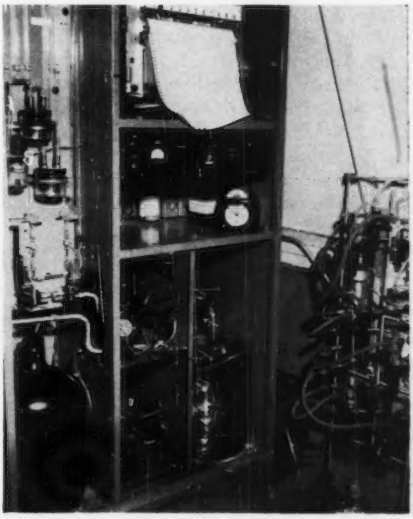

FIGURE 4. Oxides of Nitrogen Recorder

Finally, we continuously measure and record chamber concentrations of nitric oxide and nitrogen dioxide by use of the instrument shown in Figure 4. This instrument was developed by Stanford Research Institute under Air Pollution Foundation sponsorship, and our particular

model was built by Borman Engineering, Inc. Its operation is based upon color formation in Saltzman's reagent by nitrogen dioxide. In a separate gas stream nitric oxide is oxidized by ozone to nitrogen dioxide and thus the total amount of both oxides is measured in this stream. The difference between the two readings represents the nitric oxide present. This instrument has been modified in a number of ways by us to reduce its original response time from 30 minutes down to 9 minutes.

In addition to all these measurements on the pollutants in our smog chamber, we also continuously record ambient temperature and humidity inside the chambers, and light intensity outside. These measurements are made for the purpose of allowing the introduction of correction terms into the statistical analysis of the results. Beyond keeping chamber temperature within bearable limits during the summer by means of external water sprays, no attempt is made to control any of these variables.

In general, the program of experimentation with this facility involves: (a) introducing the desired pollutants into each chamber at the desired time (the presence of two separate chambers allows us to run two tests simultaneously); (b) allowing this polluted atmosphere to stand during a 100-minute period while the various analytical measurements, including the eye irritation measurements, are made; and (c) statistically analyzing the results of each experiment in conjunction with the results of other experiments in the same statistical design to draw conclusions significant at the 95% confidence level.

The pollutants we have used at one time or another during the course of this work include exhaust from the idling motor car engine, a number of pure hydrocarbons, nitric oxide, and nitrogen dioxide.

We shall now describe the results we have obtained from five series of experiments run in this facility to date. All results cited are significant at the 95% confidence level.

It was initially desired to make certain that sunlight was necessary in our facility for the eye irritation characteristic of Los Angeles smog. Therefore, our first series of experiments was run using all combinations of presence or absence of sunlight, nitric oxide, nitrogen dioxide, and idling exhaust. Results showed that eye irritation was, in fact, significantly greater in the presence of sunlight than in its absence. All subsequent experiments, therefore, have been performed in the presence of sunlight.

In the second experiment, the hydrocarbon pollutants studied were idling exhaust as such, idling exhaust plus 1-hexene, idling exhaust plus 3-methylpentane, and idling exhaust plus both of the pure hydrocarbons mentioned. Oxidizing pollutants were nitric oxide and nitrogen dioxide. These were all studied at various levels in two statistically-designed series. Results of this experiment were as follows:

1. Irradiated motor car exhaust, in concentrations similar to those found in the atmosphere, produced both the eye irritation and the oxidant manifestations of Los Angeles smog.

2. Both eye irritation and oxidant increased as hydrocarbon concentration increased up to a certain point. Above this hydrocarbon concentration, eye irritation continued to increase, but oxidant formation was actually *decreased*.

3. Some component or components of motor car exhaust caused it to be a more potent smog-former than either of the two pure hydrocarbons studied.

The third experiment comprised two statistically-designed series of runs. One of these series compared the effect of C_6 versus C_3 hydrocarbons, olefins versus paraffins, and straight-chain versus branched-chain hydrocarbons as pollutants upon eye irritation and oxidant. The other studied the effect of olefin chain length and double-bond position. Statistically significant results from this third experiment were as follows:

1. Olefins caused more eye irritation than did paraffins, and they produced more oxidant.

2. Branched-chain olefins gave more eye irritation than straight-chain olefins, and they produced more oxidant. However, chain-branching in paraffins had no effect upon eye irritation or oxidant formation.

3. Increase in olefin chain length increased eye irritation but did not affect oxidant formation.

4. Olefins with terminal double-bonds gave less eye irritation than those with non-terminal double-bonds, but there was no effect upon oxidant formation.

5. Overall, there was no significant correlation between oxidant formation and eye irritation.

The fourth experiment studied the relationship between eye irritation and oxidant formation somewhat more intensively, using 2-heptene and nitrogen dioxide at four different levels each. Results from this experiment were as follows:

1. For any nitrogen dioxide level, maximum oxidant formation occurred at a hydrocarbon level of about 120 parts per hundred million. Eye irritation, however, had no maximum in the range of concentrations studied.

2. In any given set of replicate runs, there was

a significant *negative* correlation between oxidant level and eye irritation; that is, runs giving above average oxidant level gave below average eye irritation, and vice versa.

The last experiment we want to mention here was to compare the eye irritant effects and oxidant effects of n-hexane as a typical paraffin, cyclohexane as a typical cycloparaffin, cyclohexene as a typical cycloolefin, and benzene as a typical aromatic hydrocarbon. The results of this experiment showed that none of these hydrocarbons except cyclohexene, the cycloolefin, gave significant differences in eye irritation or oxidant formation. For cyclohexene, both of these effects were significantly greater.

In conclusion, here are the things we have found out during our three years of work with this facility:

1. Motor car exhaust, in concentrations similar to those found in the atmosphere, produces both the oxidant and the eye irritation manifestations of Los Angeles smog.

2. The presence of sunlight is necessary for this effect to occur in our facility.

3. Olefins produce more eye irritation than do paraffins, and probably produce more than do aromatics.

4. Overall, no significant correlation has been found between eye irritation and oxidant formation.

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New Ventilation Manual

INDUSTRIAL VENTILATION, a manual of recommended practices, published by the American Conference of Governmental Industrial Hygienists, is now available in a new revised enlarged fifth edition. The new edition contains over 200 pages, 226 diagrams, 52 tables of ventilation material, 65 diagrams of hood designs and 12 ventilation charts. Changes in the 1958 edition are: revised equations for oven ventilation; revised equation for airflow into hoods; new material on ventilation of radioactive and high toxicity contaminants; 16 new hood designs for specific operations and 33 revised designs; new material on cleaning for radioactive contaminants; revised table for collectors in industry; new chart on comparison of air filter characteristics; revised (more complete) tables of physical constants; and additional reference material.

Industrial Ventilation combines the practical experience of ventilation engineers throughout the country and includes up-to-date information on the design and installation of industrial exhaust systems. Over 14,000 copies of the previous editions have been sold to contractors, architects and others interested in the work. A feature of the manual is a great number of line drawings and sketches showing hood types and designs. The manual has been used as a text-book and guide in industrial ventilation courses at a number of universities.

The new edition of this manual, which sells for $4.00 in single copies may be obtained from the Committee on Industrial Ventilation, P.O. Box 453, Lansing, Michigan. A hard bound copy is available at $7.00.

Air Pollution and the Public Health

Excerpts from the Harben Lectures[1] given before the Royal Institute of Public Health and Hygiene, London, Eng., May, 1957

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Population Pressures and Air Pollution

AIR POLLUTION is a problem of population growth. The population of the world attained a growth rate, best described as explosive, about 1700—somewhat after some bad famines and great pandemics had subsided. Disease exacted a fearful toll and even in the 19th century, yellow fever, cholera, smallpox, typhoid fever, influenza, and tuberculosis were great natural controls of the kind with which Malthus was concerned. (Figure 1).

None of these disasters changed the world's growth curve significantly or that of its component cities and states. Once the curve began its steep climb the trend has not deviated and no longer are disease, famine, and war the great levellers. The growth rate set by the United Nations is 11.2 persons/1000/year.

Our friends in Britain think of us in the United States as having plenty of room to expand and possibly some imagine that we are still blessed with boundless natural resources. They have a present population of 51 million and a population density of 550 persons per square mile. In the United States we had 164 million in 1956, giving us a population density of 54. But if we compare England and Wales to northeastern United States (New York, New Jersey, Pennsylvania and the New England states), we get a population of 44 million for Great Britain and 42 for us. Britain's density is 764 and ours is 251 persons per square mile. Clearly population pressure is becoming important to us.

The Demand for Energy

It is estimated that our demand for power is close to 3 per cent per annum, which must be compounded. Our record of fuel consumption in the United States is ominous. One-half of the coal we have consumed has been burned up since 1920, and nearly one-half of all the oil and gas consumed has been burned since 1940. Today we who thought we had plenty of oil must bring it in from other countries to satisfy the power demand for energy.

Putnam[2] estimates that the energy demand in 1960 in the United States will be 32 per cent for comfort heat (heating and cooling), 11 per cent for process heating, and 57 per cent for work heat. The trend in the United States is towards the use of substantial amounts of energy for cooling in hot weather. This new demand for energy is certain to increase.

In Figure 2 are shown the trends in the various fuel sources for the past hundred years in the United States. Bituminous coal is still the most used fuel. Wood has gone from the most used to the point where it is about even with oil and gas together. Burning of farm wastes, or converting them into fuels such as alcohol, will probably decrease in importance as the trend in agriculture is to return farm wastes to the soil as fertilizer. Note the comparative unimportance of hydroelectric power in terms of the total.

We will generate power soon from atomic reactors now being built. The pollution problems from atomic power are no worse than those we now have, probably they are really much less. In the event of a disaster such as a direct hit from an enemy bomb the prospect of widespread air contamination is not inviting. But there has been no new development in the history of industry that has so effectively kept ahead of the problems of public health as has the atomic energy industry. In describing the safety precautions and general safeguards on the Nautilus, an atomic submarine, Ebersole remarks that the plans are "fool-proof until a fool comes along." I suppose something of the sort applies to the projected power plants. There certainly has been no indication of neglect or failure to consider fully the possibilities of atmospheric pollution in their normal operation.

Visibility and Suspended Matter

In the London Smog* of December, 1952, the average smoke content on December 6th and 7th at stations in central London ranged from

* The word smog was coined by Dr. H. A. Des Voeux in England in 1905.

2 to 4 mg/m³, which is some ten or fifteen times the normal non-foggy value for December. At Kew Observatory it reached a maximum of 2.3 mg/m³. These values are high enough to account to the extreme dirtiness of the fogs, but they

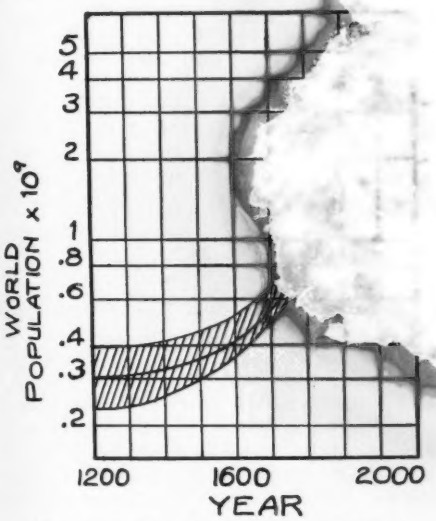

FIGURE 1. Growth of world population

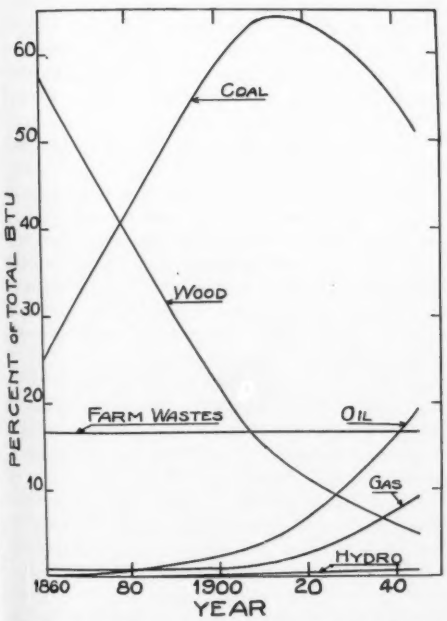

FIGURE 2. The fuel resources of the United States.

not account for the low visibility. According to Douglas and Stewart,[3] visibilities as low as five yards—as in a London "Pea-Souper"—need suspensions of about 200 mg/m³ and they suggest that some 98 per cent of such a concentration would be water droplets blackened by suspended matter. The size of these fog droplets and visibility of the suspension depends on local

ve been in Los Angeles when their eye-smarting smogs is about, as visibility, by lon is fairly good. If, by y, Los Angeles should sibility would unquesand the toxicity could low to very high. characteristic of Los are they wet. Los the busiest we have. es visibility but, by ondon not dirty.

Temperature Inversions and Air Pollution

In 1924 Shaw and Owens[4] made tests to determine the lower limit of "ceiling" of inversion layers over the Croydon aerodrome (near London) during a severe December fog. On the first night their test kite came into brilliant moonlight at 300 feet. A little later the inversion layer moved up to 500 feet and then to 900. At the same time the temperature at Kew Observatory was 34°F and persisted up to 300 feet. At 400 feet it had risen to 45°F and so remained up to 1,250 feet when cold air was encountered.

During the 1952 fog in London the photograph shown in Figure 3 was taken rather by chance and shown in *The Times*. Ranmore Common is only 615 feet above sea level yet is obviously above the fog. At other places the ceiling was sometimes higher and sometimes lower. In their preliminary report. Douglas and Stewart state that the "fog was thickest in the London Basin in which visi-

FIGURE 3. Box Hill, Surrey, looking towards Ranmore Common, alt. 615 feet, during the London fog of December 1952. (Courtesy A. F. Kersting, London)

bility over large areas was below 20 yeards for many hours on end and was often below 10 yards."

Shaw and Owens, studying London fogs in 1924, recount the fact that their test balloons could be seen to bounce downwards at the edge of the inversion layer and to remain for a few moments until the gases within the balloon and in the surrounding air came into equilibrium, then the normal ascent would continue. This observation has been noted and rediscovered frequently, for the stability of such inversions and their ceilings are now recorded routinely in many large cities.

Fog Disasters

The fogs along the Meuse Valley of Belgium in 1930, at Donora, Pa. in 1948, and in London, 1952, all came in areas accustomed to such visitations. They differed from previous episodes mostly in the fact that they lasted for 4 to 5 days. Had they lasted only one day, it is likel that they would not have posed a health problem. As they occurred each was a major disaster. They differed one from the other only in the numbers of persons killed or made severely ill. Mortality and morbidity rates were comparable in all three episodes, the totals in each case varying with the populations affected. Meuse Valley came first. It is of interest that one of the Belgian authorities commenting upon it later said that a comparable episode in London might kill 3200 persons—the '52 fog in London killed about 4000 within the week and some 8000 deaths in excess of the expected occurred over the next two months.

The precise etiology has not been determined for any one of the three and it is unlikely it ever will be. This is one of the many examples of problems in public health in which it is easier and far more practical for engineers to control effluents than it is for toxicologists to isolate and identify the specific culprits. I cannot imagine a recurrence of the Donora incident while London, with the most serious problem of all, is now a very different place in terms of pollution control, than it was in '52.

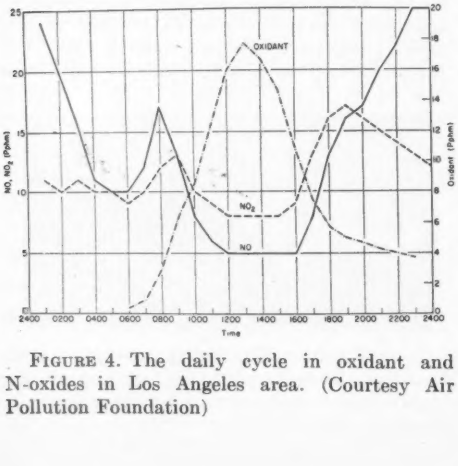

FIGURE 4. The daily cycle in oxidant and N-oxides in Los Angeles area. (Courtesy Air Pollution Foundation)

In all three of these episodes the air was wet, it had a peculiar sulfurous smell and it dirtied everything. The temperature was not far above freezing. Of Donora they said, "there was something white and scummy mixed up in it." The asthmatics and cardiacs were particularly susceptible in all three fogs. The older people were more bothered than the young but no real immunity by any age group was apparent.

Los Angeles Smog

If one likes to live in climatic order and beatific monotony Los Angeles, for most of the year, has much to recommend it. The New Englander gets restive under such a regime and looks for a bit of nasty weather to relieve his boredom. I would not trade anything Los Angeles has for the four seasonal changes that are an annual occurrence in New England—and they are usually ushered in and out with some thoroughly bad weather.

Los Angeles has never had pollution episodes like those of Meuse Valley, Donora, or London. If one can rely upon nature to keep repeating its weather patterns, there is no liklihood that they will get such a visitation. Their health statistics are impressive as are those for the entire state of California.

An important feature is the fact that Los Angeles smogs damage vegetation.

The smog irritant is now strong enough to make people move out into the country. Many remember ten or fifteen years ago when they had no such bad eye irritant; protests have been mounting in vigor and in acrimony. Undeniably it is affecting the people's way of life and in a land of plenty this is not to be endured with equanimity or complacence.

It was accepted, certainly well before 1950, that Los Angeles smog had unusual oxidizing power. The presence of ozone in smoggy air was proved by collecting it on silica gel, releasing it, and identifying it spectroscopically.

Smog can be produced by irradiating various compounds and NO_2 either by sunlight or by blue fluorescent light. Visible aerosol formation results from irradiating 3 ppm of hexene and 2 ppm of NO_2 in 5-liter flasks, transparent for 3660 Å, and is produced most easily with ring compounds such as cyclohexene.

As rubber is very sensitive to ozone, it was found that bent rubber strips in the test flasks gave a convenient way of determining ozone by the depth of the crack produced in a given time. The irradiations are done in air or oxygen and the light source is either sunlight or blue fluorescent lights.

The Daily N-Oxide Cycle in Los Angeles

A recent report by Faith et al.[5] shows the N-oxide daily cycle in Los Angeles (Figure 4). The oxidant (smog former) reaches its daily peak around noon, when solar radiation is maximum, the N-oxides are at their low at noon and highest at night. The oxidant needs sunlight for its formation while the N-oxides do not.

The Control of Motor Car Exhausts

In the following table are shown typical analyses of modern automobile exhaust gases.[6]

	Idling	Acceleration	Cruising	Deceleration
Hydrocarbons (C_3-C_9), as hexane, ppm	1275	410	354	5125
Acetylenic, as acetylene, ppm	825	18	64	687
Oxides of nitrogen, as NO_2, ppm	8	4180	1606	18
Lower aldehydes, as formaldehyde, ppm	88	1369	264	193
CO, %	3.6	0.0	0.4	1.5
CO_2, %	10.0	13.7	12.9	6.1
O_2, %	1.4	1.3	1.1	9.5

It is to be noted that carbon monoxide, CO, goes up during idling while nitrogen oxides drop. During acceleration and cruising, combustion is at its best with result that CO is then lowest, CO_2 is highest, and NO_2 has fallen. The values for the unburned hydrocarbons are also significant in relation to possible control of exhaust gases.

No nation has paid attention to N-oxides in chimney gases. The concentrations increase with furnace temperatures—the better the plant is run the more N-oxides we get.

Pollution by Sulfur Dioxide

Smelters treating sulfide ores get rid of the sulfur by roasting it off. The stack effluent will contain SO_2, therefore, in varying concentrations. If SO_2 exceeds about 2 per cent, the gas can be cleaned and the SO_2 converted into sulfuric acid. Another possibility is to purify the effluent, collect the SO_2 and compress it to the liquid. A third is to treat the effluent gas with a cheap reducing agent, such as natural gas, and recover elemental sulfur of high purity. The fourth possibility and the one most frequently used until about 1930

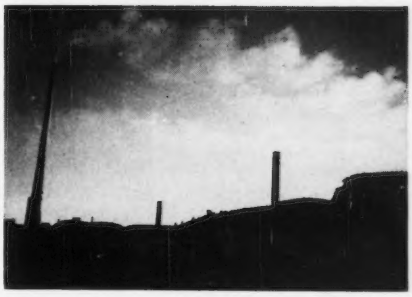

FIGURE 5. Smelter stacks, 300 and 600 feet high, with plumes going in different directions. (Courtesy Messrs. Olson and Abersold, American Smelting and Refining Co.)

was to discharge effluents through high stacks, many of which today exceed 600 feet.

But the weather aloft does not always do what one on the ground would like. I show in Figure 5 two smelter stacks, one of about 300 feet and near it a new one over 600 feet. The smoke plumes from the two are going in opposite directions.

I would like to turn to a brief consideration of the indirect role of sulfuric acid in abating some major pollution problems in the non-ferrous smelting business—this acid is the most important and most used heavy chemical manufactured today. It is relatively cheap, handled and shipped in bulk, and used in large amounts by the steel, petroleum, fertilizer, and other operations all over the industrial world. With the growth of the chemical fertilizer industry major smelters, like Trail, found a steady market for sulfuric acid.

Farmers with an abundance of new land are not apt to be fertilizer-minded. If they have been careless and impoverished their land, or when they must increase their yield, they will heed advice from agricultural experts. When the Trail Smelter decided to enter the phosphate fertilizer business, as an outlet for their sulfuric acid, they had to do some active campaigning and teaching of their prospective customers. They gave them fertilizer and demonstrated, on the farmer's own land, the improved yield that fertilizer would bring about. Today their acid and fertilizer business constitute major items in the smelter's economy. (Figure 6.)

Modern British power stations, burn bituminous coal containing about 1 to 1½ per cent sulfur. Our plants in the east burn soft coal containing about ¾ per cent sulfur while soft coal in our mid-west and in the Rocky Mountains may exceed 5 per cent sulfur. In any one of these large plants burning high sulfur coal the amount of SO_2 evolved compares with that from smelters.

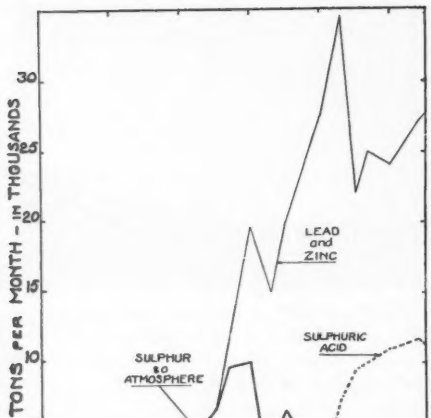

FIGURE 6. Production of sulfur gases, sulfuric acid, zinc and lead at Trail Smelter. (Courtesy Consolidated Mining Corporation)

The oil refineries in our west, particularly those in the Los Angeles basin, began desulfuring petroleum in their refineries of their own volition well before the public looked askance at SO_2 emissions. Since oil and natural gas are the only fuels burned in Los Angeles, the elimination of sulfur from petroleum products is a significant step. There is every indication the practice will spread. By desulfuring, the refineries capture sulfur which they dispose of to chemical manufacturers for conversion into sulfuric acid and resale to the refineries.

It is doubtful if this step has actually lessened smog formation in Los Angeles. Sulfur dioxide is

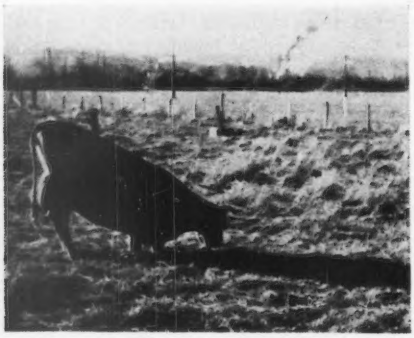

FIGURE 7. Grazing cow with fluorosis. (Courtesy Dr. Charles R. Williams)

mildly reducing and the low concentrations found in Los Angeles in smogs, 0.1 to 0.2 ppm, are enough to counteract in a measure the oxidant effects of nitrogen oxides, peroxides, and ozone.

Industrially, SO_2 is not an especially troublesome gas. Certainly it is not a dangerous gas. It gives a useful and convenient way to follow the gaseous emissions from stacks more or less as on follows B Coli in water supplies. It is not a normal constituent of air and if present the evidence points towards pollution. "Some circumstantial evidence is very strong, as when you find a trout in the milk." (Thoreau)

Pollution by Fluorides

The effluent fume from modern plants making aluminum contains fluoride salts, silicon tetrafluoride, and some hydrofluoric acid much of which can be deposited on vegetation at distances up to several miles. If animals graze there, sooner or later they come down with fluorosis which usually first affects their teeth and then various of their bony structure. In dairy cattle there follows a reduction in milk and butter fat.

In Figure 7 is shown a photograph taken by my colleague, Dr. Charles R. Williams, of a cow kneeling on her forelegs and grazing. In the background is the offending stack. The cow's milk production has dropped; she has developed exostosis; she is suffering from malnutrition and her days obviously are numbered. Damage to cattle in this particular area resulted in claims by the farmers for fluorosis of their herds, loss of milk production, damage to flowers (especially gladioli) and to fruit trees. The incident occurred in our coastal northwest where rain is plentiful, snow is unusual, and the grass is always green.

In making iron and steel, large quantities of fluxes like limestone and dolomite are used. Supplies must be conveniently near to keep down costs. In parts of the United States the fluxing rock may contain fluorospar which can cause fluoride pollution from the furnace effluents. Stack gases from the steel mills are in such huge volume that their economical cleaning poses a very difficult problem.

We are not rid of fluorine pollution. We know we must clean large volumes of gas, and have no hopeful indication of a reasonable return on the investment. It is not a pleasant prospect.

The Effect of Sulfur Dioxide on Plants

Because its occurrence as a pollutant is so common the effects of SO_2 on plant life have been carefully and thoroughly studied. In practice we see both acute and chronic leaf damage from SO_2. Typical acute markings are characterized

by sharply defined marginal areas killed. These will dry up and usually bleach to a sort of ivory color while around the injured part the leaf appears healthy and normally green. This is the type of injury following a sudden fumigation which need last only an hour or so.

Chronic or chloritic markings from SO_2 follow prolonged exposure to much lower concentrations than cause the acute injury. The leaf does not collapse but the damage is identifiable by histological examination and photosynthesis can be reduced to about half normal.

The Effects of Los Angeles Smog on Vegetation

The characteristic lesions from Los Angeles smog are identifiable by skilled plant pathologists and have been described and illustrated fully. According to Went they were of comparatively recent origin—say 1944—and seemed to be associated with the density of motor traffic and especially with increasing use of high octane fuel.

Went found the same kind of plant damage in other large cities—San Francisco, New York, Philadelphia, London, Manchester, Paris, and Sao Paulo—but did not find it in Houston, Amsterdam or Rome. In Paris and New York it began about 1952, he though, and he suggests that the introduction of high octane fuel might account for it.

In Figure 8, are Went's estimates of gasoline consumption from 1947 to 1954. The dotted line, at 12 tons per day, indicates about where plant damage begins to be seen. Note that there are two lines for London since the fuel consumption figures available were for the area of 680 square miles so he assumed that either one-half or two-thirds would have been used in the central London area of 120 square miles.

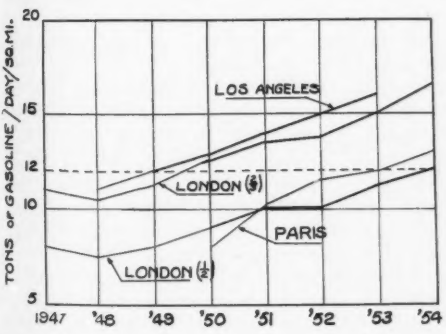

FIGURE 8. Gasoline consumption and evidence of smog damage to vegetation in Los Angeles, London and Paris. (Courtesy Dr. Frits Went)

This is another example of my same general theme—an increase in pollution to objectionable levels follows uncontrolled increase in human activity.

Discussion

There are many economic advantages for an industrial nation to have large cities and large industrial units. We claim that our industrial success is due in great measure to our national tendency to standardize on fewer types of products than is done elsewhere. Such standardization is adapted to use of large units.

Most of us admit that small automobiles are a lot more convenient for city use than our modern monsters that scarcely fit into garages built a few years ago. We could easily turn to the small cars in preference to the large, but if we do, what assurance have we that our city traffic problem would be lessened.

All large cities are struggling with parking problems and the only solution at present is to make illegal parking a severe offense with severe fines. But can you think of anyone, certainly any American, who would not be ready occasionally to thwart such a rule, if he though he could get away with it? No car, large or small, is penalized for its exhausts, yet Los Angeles has proved to the satisfaction of most of us that such penalization or regulation will have to come very soon.

No power plant, large or small, is obliged or even requested to do anything to reduce its N-oxide effluents. We watch their particulate emissions, the blackness of their plume, its opacity (black or white), we pay attention to SO_2 emissions, and because of their significance in combustion measurements we record CO and CO_2. How many measure or record N-oxides? Very few.

The administration of inspection schemes for appraising automobile or power plant exhausts could become expensive, burdensome and bureaucratic to the point they would be wholly impracticable. It is likely that improvements and changes in the engines of motor vehicles will be accomplished before we have successfully coped with traffic and parking problems.

In the practice of industrial hygiene we control objectionable air contaminant to reasonable levels. We rarely are able to get rid of them entirely. This applies to community pollution problems. They are accentuated by population pressures. They may be expensive to control because the materials recovered are rarely of value. This is a state of affairs which has always been with us and it will continue to be with us in the future.

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X-ray Analysis Conference

THE SEVENTH Annual Conference on Industrial Applications of X-ray Analysis will be held August 13–15, 1958 at the Albany Hotel, Denver, Colorado. A wide field of technical papers will be presented in an informal atmosphere with ample time for duscussion. Fields to be covered are x-ray fluorescence, diffraction, absorption, and instrumentation.

Address inquiries to William M. Mueller, Metallurgy Division, Denver Research Institute, University of Denver, Denver 10, Colorado.

Radiological Health

THE U. S. Public Health Service has established a new Division of Radiological Health in the Bureau of State Services. This Division will provide technical assistance to state agencies dealing with medical, industrial and other activities involving public exposures to radiation, and will conduct varied research and training programs. The problems of radiation in air, water, milk, and food will be included. Dr. Francis J. Weber will assume duties as Chief of the Division about July 1, 1958.

Urinary Arsenic Levels as an Index of Industrial Exposure

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Industrial Hygiene Foundation, Mellon Institute, Pittsburgh, Pa.

THE MOST common procedure used to evaluate environmental exposures is the determination of the concentration of the contaminant in the air in the approximate breathing zone of the worker. The limitations of air analyses are well recognized and are frequently supplemented by analyses of biological specimens, particularly the urine. Tests have been developed based on the determination of the contaminant itself, its metabolic products or changes in normal constituents of the body. The results provide a measure of absorption and thus give an index of the magnitude of exposure but are not necessarily proof of poisoning. The usefulness of biochemical tests depends upon the quantitative correlation which can be established between the concentrations found in the biological specimens and the magnitude of the exposure.

Urine analyses are commonly used for evaluating exposures to metallic elements. In using such biological tests the base line is the so-called normal levels of the constituent in question. In other words, the concentration which is present in persons who have not had industrial or other unusual exposure to the element. A good correlation between the concentration of lead in the urine and the magnitude of exposure has been established. But in the case of arsenic the relationship has not been placed on a definite basis. One reason for this is the fact that there are many organic arsenic compounds which have a relatively low order of toxicity as compared to inorganic compounds and the level of urinary excretion may vary widely in relation to potential physiological effects. Another factor which may be of real significance is the arsenic content of certain foodstuff and it is the purpose of this paper to discuss this aspect.

In a pilot plant operation in which there was a possibility that arsine could be generated, a program of air and urine analyses was conducted. The air analyses indicated that control measures were operating effectively as the results showed arsenic either absent or present only in trace amounts at the breathing zones of the workers. However, in the course of the urine analyses, occasional values exceeding 0.5 milligrams of arsenic per liter of urine were found. In the cases in which high arsenic was found in the urine there was no evidence whatsoever of any adverse effects. The question then arose as to the source of the arsenic.

A careful check of the air analysis procedures and sampling locations confirmed previous findings that exposure in the pilot plant was not a significant source of arsenic. A check of all other plant areas did not reveal any points of potential exposure to arsenic by the personnel in the pilot plant. Medication was also ruled out. The question of arsenic in foodstuff was then raised.

The use of lead arsenate as an insecticide spray on fruits and vegetables was considered. However, the levels of arsenic excretion in orchardists and consumers of sprayed fruit reported by the U. S. Public Health Service[1] indicated that fruits and vegetables were not the main source of arsenic in the pilot plant personnel. On the other hand, Monier-Williams[2] pointed out the high level of arsenic in sea food. Oysters were found to contain 3 to 10 ppm arsenic and some shellfish, particularly mussle, as much as 120 ppm. Values as high as 170 ppm were found in prawns, a crustacean. After eating lobster containing 70 ppm of arsenic, the urine was reported to contain as much as 11 ppm of arsenic.

Attention was therefore directed to sea food in the diet of personnel in the plant. It was early evident that urinary levels of arsenic were markedly influenced after eating sea food and that concentrations in excess of 0.5 mg per liter were not uncommon.

Urinary Arsenic Levels in Plant Personnel

During the course of this study approximately 1000 determinations were made of arsenic in the urine of persons in the pilot plant and other plant areas. After destruction of organic matter, the arsenic was isolated by volatilization as arsine and determined by the molybdenum blue method.[3] The results are reported as determined and were not corrected for specific gravity as

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TABLE I

Urinary Arsenic Levels in Pilot Plant and Other Plant Personnel During the Period March 5 to June 16, 1957

Subject	Total Number Samples	Arsenic in Urine, mg/liter		Per cent Less Than 0.10
		Range	Average	
A	23	0.04 to 0.14	0.08	74
B	28	0.02 to 0.11	0.06	79
C	25	0.02 to 0.30	0.10	64
D	42	0.02 to 0.24	0.08	69
E	41	0.04 to 2.00	0.22	44
F	29	0.02 to 0.11	0.06	90
G	44	0.02 to 1.10	0.12	66
H	38	0.04 to 0.42	0.09	84
I	43	0.02 to 0.19	0.07	84
J	41	0.04 to 0.25	0.09	66
K	42	0.04 to 0.27	0.08	81
L	41	0.02 to 0.36	0.07	83
M	40	0.03 to 0.14	0.08	78
N	40	0.02 to 0.29	0.07	83
O	27	0.04 to 0.09	0.05	100
P	4	0.04 to 0.06	0.05	100
Q	26	0.03 to 0.28	0.06	96
R	24	0.04 to 0.14	0.07	92
S	23	0.04 to 0.13	0.07	87
T	20	0.04 to 0.18	0.08	75
U	22	0.02 to 0.10	0.05	86
V	17	0.04 to 0.12	0.07	82
W	7	0.04 to 0.15	0.09	43
X	17	0.02 to 0.12	0.06	94
Y	17	0.04 to 0.10	0.05	94
Z	10	0.02 to 0.25	0.07	90
AA	12	0.02 to 0.09	0.05	100
BB	7	0.04 to 0.09	0.05	100
CC	6	0.04 to 0.12	0.08	67
Total	756	0.02 to 2.00	0.08	79

the validity of this procedure has not been established for the urinary excretion of arsenic.

Table I gives the results of the analysis of 756 specimens collected from 29 persons during the period March 5 to June 16, 1957. This table does not include a number of results where it was known that the person had eaten sea food. On the other hand, some values are included which were undoubtedly influenced by eating sea food but before it was recognized that this might be a factor and hence no record was kept of the diet. The results of these determinations range from 0.02 to 2.0 mg/liter. The second highest value was 1.10 and the third highest 0.42 mg/liter. The average concentration for all determinations was 0.08 mg/liter. The lowest average concentration for an individual was 0.05 and the highest 0.22 mg/liter, the second highest being 0.12 mg/liter. Seventy-nine per cent of all the values were less than 0.10 mg/liter. These results indicate that in the absence of unusual amounts of arsenic in the diet or other exposure to arsenic that the urine level in the majority of cases may be expected to be less than 0.1 mg/liter.

A comparison was next made of the urinary arsenic levels of groups of employees eating various kinds of sea food and those not eating sea food. The results of these determinations are given in Table II. The sea food eaten included clam chowder, halibut, lobster tail, shrimp, salmon, scallops, crab meat and various types of fish. In every instance there was an increase in the urinary arsenic level after eating sea food. The average pre-test values in the

TABLE II

A Comparison of Groups of Employees Eating Various Kinds of Sea Food and Those Not Eating Sea Food

Date	People Eating Sea Food					People Not Eating Sea Food		
	No.	Sea Food	Conc. of Arsenic as As in Urine, mg/liter			Conc. of Arsenic as As in Urine, mg/liter		
			Pre-Test	Range	Average	No.	Range	Average
5-17	3	Clam chowder	—	0.41–0.62	0.52	15	0.06–0.16	0.09
5-24	3	Halibut	—	0.20–0.38	0.29	20	? –0.14	—
5-28	2	Lobster tail	—	1.20 & 1.50	1.35	18	? –0.10	—
6-7	1	Fish—lunch & dinner	0.06	0.70	—	—	—	—
6-21	4	Fish	0.05	0.11–0.14	0.13	11	0.02–0.07	0.05
6-28	5	Fish	0.04	0.12–0.36	0.22	11	0.04–0.08	0.05
7-5	7	Shrimp or fish	0.04	0.05–0.16	0.12	8	0.04–0.05	0.04
7-12	5	Lobster or salmon	0.07	0.08–0.25	0.13	10	0.04–0.07	0.05
7-26	5	Scallops or fish	0.05	0.09–0.26	0.17	9	0.04–0.06	0.05
8-2	2	Fish	0.05	0.15–0.15	0.15	9	0.04–0.12	0.07
8-9	7	Crab meat or fish	0.05	0.05–0.48	0.21	8	0.02–0.09	0.06

TABLE III

Urinary Arsenic Levels After Eating Various Kinds of Sea Food

Arsenic in Urine as As, mg/liter

H.H.S.—Halibut		L.S.—Filet of Sole		B.S. Various Kinds of Sea Food			
Time	mg/liter	Time	mg/liter	Date	Fish	Time	mg/liter
Pre-test	0.024	Pre-test	0.12	9–16		Pre-test	0.016
4 hrs.	0.080	3½ hrs.	0.66	1:00 pm	White fish	4 hrs.	0.056
10 hrs.	0.081	11 hrs.	0.78	6:00 pm	Salmon loaf	8½ hrs.	0.058
19 hrs.	0.064	18 hrs.	0.26	9–17			
23 hrs.	0.050	24 hrs.	0.15	7:00 am	Tunafish	14 hrs.	0.060
50 hrs.	0.036	49 hrs.	0.14	12 Noon	Scallops	22 hrs.	0.21
				6:00 pm	Halibut	28 hrs.	0.15
						34 hrs.	0.39
						43 hrs.	0.31
						50 hrs.	0.11
						72 hrs.	0.11

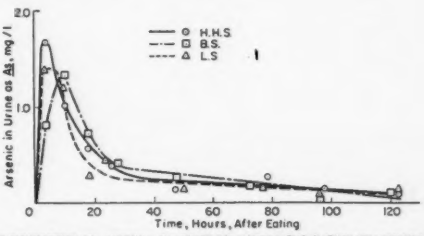
groups eating sea food range from 0.05 to 0.09 mg/liter and the average after eating sea food range from 0.13 to 1.35 mg/liter. The two highest average values 1.35 and 0.52 mg/liter were observed after eating lobster tail and clam chowder respectively. The average values for the groups not eating sea food range 0.06 to 0.09 mg/liter. The wide variety of foods eaten by the groups not eating sea food in conjunction with the data presented in Table I indicates that sea food is the main source of arsenic in the diet.

Urinary Arsenic Levels in Persons Having No Plant Exposure

Two additional experiments were conducted in which the urinary arsenic levels of three subjects who had no plant exposure were studied. The results of one experiment are given in Table III. It will be noted that after eating halibut there was a slight increase over pre-test values but the levels remained within the normal range. However, after eating filet of sole there was a marked increase of about six fold in the urinary arsenic level as compared to the pre-test value. The third subject, after eating various sea foods at consecutive meals, showed only slight changes in urinary arsenic after white fish, salmon loaf, and tuna fish. However, a moderate increase was noted after eating scallops and halibut. In the other experiment the same three subjects ate lobster tail for lunch. Urine specimens collected four hours after lunch showed 1.68, 1.40 and 0.78 mg of arsenic per liter. (Pre-test results were 0.01, 0.05 and 0.03 mg/liter respectively). Samples collected 10.5 hours after lunch showed a decrease to 1.02 and 1.19 mg/liter in the first two subjects and an increase to 1.32 mg/liter in the third subject. After approximately 24 hours the values were 0.39, 0.44 and 0.39 mg/liter and at 48 hours, the values were approaching the normal range. Figure 1 shows graphically the results of this experiment. Not only are the high levels of arsenic of interest but also the rapidity with which it is excreted. It is interesting to note that Bang[4] in 1916 reported great daily variations in arsenic excretion and found that the highest amounts were found after a fish diet (0.78 mg/liter).

Urinary Arsenic Levels as an Index of Industrial Exposure

The value of urinary arsenic determination as a measure of industrial exposure to arsenic is controversial owing to the fact that no definite relationship between urinary arsenic levels which may be considered normal and those which are indicative of poisoning has been established.



CONCENTRATION OF ARSENIC IN URINE FOLLOWING THE EATING OF LOBSTER

Figure 1

Webster[5] reported the urinary arsenic level in 18 children having no known exposure to arsenic to range from 0 to 0.065 mg/liter with an average of 0.014 mg/liter; and for 26 adults with no known exposure the corresponding figures were 0 to 0.060 and 0.015 mg/liter respectively. Watrous and McCaughey[6] reported a range of 0.04 to 0.21 mg/liter with an average of 0.13 mg/liter in a random group of applicants. Pinto and McGill[7] analyzed 147 urine samples from 124 individuals working in industry but having no known exposure to arsenic. The results range from 0 to 2.06 mg/liter; the median value was 0.10 and the average 0.13 mg/liter. Eighty-eight per cent of the samples had less than 0.2 mg/liter.

These results in conjunction with the results given in this report indicate that in un-exposed persons one may expect the urinary arsenic level in the majority of persons to be less than 0.1 mg/liter with a few values in excess of 0.20 mg/liter unless there is some specific arsenic intake as for example in the diet.

These results also demonstrate that high urinary arsenic levels may occur with no evidence or indications of any adverse affects. In the *Queries and Minor Notes*[8] it is stated: "In general, it appears tenable that any value of arsenic in urine to a peak near 0.7 milligrams per liter may be regarded as within normal limits although possible exceptions may be taken to this statement. Higher values, up to 3 milligrams may be regarded as representing an intake of arsenic unusual and undesirable except in connection with arsenical therapy." Pinto[8] stated "Even with urinary arsenic levels of 4 or 5 mg/liter we have seen only one doubtful case of systemic sickness." Elkins[9] suggests a maximum allowable concentration of 1 mg/liter of arsenic in the urine for exposure to arsenic trioxide and 0.5 mg/liter for exposure to arsine.

It is evident, therefore, that urinary arsenic values do not provide a reliable index to industrial exposure, as no definite relationship has been shown between urinary arsenic levels and evidence of poisoning. On the other hand urinary arsenic levels in a group of exposed persons may serve to check the efficacy of control measures and indicate if excessive absorption of arsenic occurs. However, the occurrence of a high value in an individual is not evidence of industrial exposure. Such findings should be checked to ascertain if the excessive absorption is due to industrial exposure or to some outside source, particularly from sea food in the diet.

Acknowledgments

The authors wish to acknowledge collaboration of the Research Department, Zinc Smelting Division, St. Joseph Lead Company, whose pilot plant operations provided the basic data recited herein. Specific credit is due Research Superintendent, Robert E. Lund, who first recognized and then proved the true origin of urinary arsenic in the circumstances proved.

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Dust Control in Handling Refractory Brick

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IN THE last dozen years, occasional reference has been made in the literature to real or potential exposure of silica brick workers to dust inhalation and subsequent pneumokoniosis. The evaluation of the problem has ranged from the finding of a mild hazard[1] in furnace lining operations to a fairly severe hazard[2, 3] in making refractory brick. Even where the potential seemed small, a program of dust control and periodic medical evaluation was recommended.

Some of the dust problem from furnace lining has been partially solved by process control, i.e. use of special shapes of brick to replace brick cutting, or by use of ventilated saws when cutting was necessary.[4, 5] The same industry which utilized these procedures suggested an additional desirable precaution for furnace lining work,[6] namely that silica brick be surface-treated to bind loose particles to the surface or otherwise diminish the dust dissemination in handling and laying up furnace refractories. This was accomplished on a plant scale by dipping full pallets of silica brick into a tank of aqueous binder solution. No restacking or special handling of the pallet was necessary.

The agent of choice was an ammonium lignin sulfonate by-product of the paper industry.* This water soluble material was found to be not only feasible in use, but capable of eliminating visual evidence of dust dissemination when bricks were dipped into a solution containing one pound of the chemical per gallon of water, i.e. about twelve per cent concentration. The presently reported experimental work was undertaken to evaluate further the dust dispersal from handling standard refractory silica brick, surface-treated with various dilutions of this material. The experiment was designed to determine comparative amounts of air-borne dust of hygienic importance resulting from handling brick in a simulated series of furnace lining maneuvers, the brick being untreated, treated with water, and treated with ammonium lignin sulfonate dissolved in water at concentrations of 0.25, 0.5, and 1.0 pound per gallon.

The bricks used were regular duty 9-inch straight silica brick, packed 504 pieces in a strapped pallet. These were received new, and for the experiment, only bricks from the interior of the pallet were used. The ammonium lignin sulfonate (Orzan A) was obtained from a distributor, packed in fifty-pound bags.

For each test, forty bricks were placed, ten at a time, in a wooden rack, six bricks on end, three on edge, and one on its flat side; dipped four to five seconds in a tank containing about 37 gallons of the treating liquid; drained five minutes and placed in an air-circulating drying oven. Twenty bricks at a time were dried fifteen to sixteen hours at 120°–128°F. Figures 1 and 2 show some of these procedures. Although drying the brick is not necessary in large scale practice, it was done to insure that variable quantities of moisture did not affect experimental results.

Experimentally it was found that 4–5 seconds immersion gave about ½ inch penetration of solution into bricks as shown in Figure 3. Forty bricks absorbed about two gallons of liquid under these conditions. For the tests, the dried bricks were cooled to room temperature.

The only variation from this procedure was in Run 1 when forty bricks were transported with minimum handling directly from the pallet to the test room. The five runs were as follows:

Run 1—no brick treatment
Run 2—1.0 pound chemical per gallon (about 12% by weight)
Run 3—Water treatment
Run 4—0.5 pound chemical per gallon (about 6% by weight)
Run 5—0.25 pound chemical per gallon (about 3% by weight).

The comparative test runs were made in a tightly closed and unventilated chamber of about three hundred cubic feet volume, its dimension being 6.5 x 5.0 x 9.1 feet. About one-third of the interior surface is glass, the rest being painted wood, plaster and asphalt tile. Before a test run the chamber was thoroughly vacuum cleaned and wet-dusted. As far as possible, all test runs were made in the same manner, the same operator repeating the same operations in each case to insure uniformity of technique. This practice extended to brick laying, dust sampling, dust counting, and subsequent size observation.

After forty prepared bricks were stacked in

* Orzan A from Crown Zellerbach Corp., Camas, Washington.

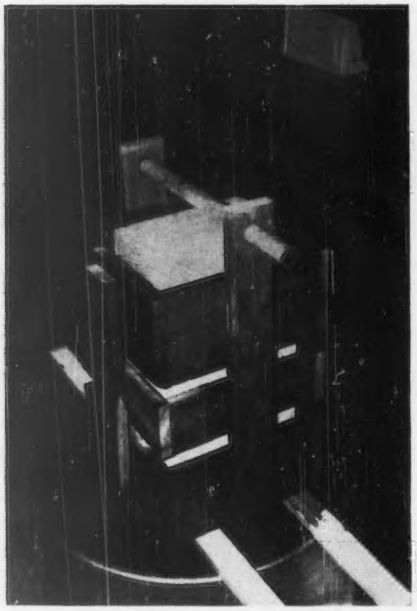

FIGURE 1. Dip rack and tank

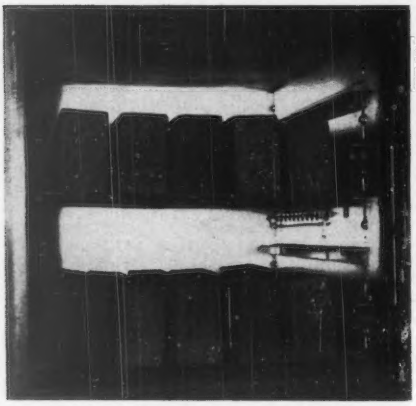

FIGURE 2. Brick drying for experimental run

the test chamber, temperature and moisture content of the air were determined, the room ventilation was blanked off completely, and the air sampling equipment was connected. During one hour the bricks were handled in a prescribed series of simulated dry wall laying-up operations illustrated in Figure 4. The simulated walls were laid on a wooden surface at table height. The standardized maneuvers were as follows:

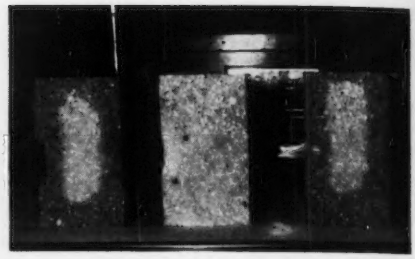

FIGURE 3. Cross section through dipped brick (right and left) and untreated brick (center).

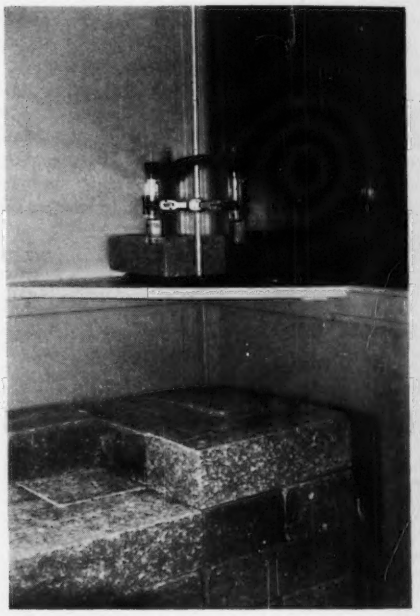

FIGURE 4. Dust sampling station and section of one simulated wall from interior of dust room.

1. 9″ wall; side 1 up, 5 forward*
2. 9″ wall; side 2 up, 5 forward
3. 9″ wall; side 3 up, 5 forward
4. 9″ wall; side 4 up, 5 forward
5. 9″ wall; side 1 up, 6 forward
6. 9″ wall; side 2 up, 6 forward
7. 9″ wall; side 3 up, 6 forward
8. 9″ wall; side 4 up, 6 forward
9. Random stacking
10. 13½″ wall; random selection of sides 1 and 2 up

* Sides 1 and 2 are flat 9″ x 4½″
Sides 3 and 4 are edges 9″ x 2½″
Sides 5 and 6 are ends 4½″ x 2½″

TABLE I
Typical Test Protocol
Test Run No. 3 Date 8/7/57

3:00 p.m.	w.b. 68.9°F. d.b. 74°F.
3:13	Start maneuver #1 and samples 5a, 5b
3:20	Start maneuver #2
3:25	Start maneuver #3
3:31	Start maneuver #4
3:37	Start maneuver #5
3:43	Stop samples 5a, 5b
3:44	Start maneuver #6 and samples 6a, 6b
3:49	Start maneuver #7
3:50	Incipient fogging*
3:54	Fogging definite*
3:55	Start maneuver #8
4:01	Start maneuver #9
4:07	Start maneuver #10
4:13	Complete maneuvers
4:14	Stop samples 6a, 6b
4:15	w.b. 75°F. d.b. 77.5°F.

*In each run, condensation occurred on interior walls of test room after 40–45 minutes, indicating tightness of room. Saturation of room air did not occur in any test run. The only source of heat and moisture in the room was the brick manipulator, all lights, pumps, etc., being outside the room.

Thus each brick received repeated handling; a total of 400 handlings occurred in an hour, a rate well above that likely to be practiced in furnace lining. A typical time protocol of a test run is shown in Table I.

During these laying-up operations, duplicate midget impinger samples at a meter-controlled sampling rate of 0.1 cubic foot per minute were taken at a fixed head-height location in the test room (Figure 4). The sampling position was within three feet of all bricklaying operations. A pair of samples was taken during the first 30 minutes of a test run. At mid point of the test a second pair of samples was started and run for 30 minutes. At the end of a test run psychrometric readings were again made.

Impinger samples were evaluated by direct light-field microscopy using a 10X eyepiece with Whipple disk and 10X (16 mm) objective, (N.A. = 0.25). The counting cell was one millimeter deep. When necessary, dilutions were counted rather than the original sample, with double distilled water used throughout for sampling and dilutions. All results are the average of five individual counts, each slide being observed after twenty minutes settling. The limit of resolution of the microscope system is about one micron, and only particles less than five microns were counted. Very few larger particles were observed. The dust counts and psychrometry are shown in Table II.

After the impinger samples were counted, the solids from them were concentrated by centrifuging at about 800 G. for fifteen minutes. An aliquot of the combined concentrate of four impinger samples per test run was dispersed on a clean slide and the size of at least 250 particles per run was observed microscopically with filar micrometer eyepiece and 44X (4 mm) objective (N.A. = 0.65).

Data from these measurements were analyzed graphically with the following results:

Run #	Brick Treatment	Logarithmic mean-microns	Standard Deviation
1	None	1.9	1.7
2	1 pound/gal	1.4	1.9
3	Water dip	1.3	1.6
4	0.5 pound/gal	1.2	1.6
5	0.25 pound/gal	1.4	1.6

Discussion

From Table II several points are evident concerning brick treatment with ammonium lignin sulfonate. Most notable is the significantly lower dust concentration when comparing the series of

TABLE II
Summary of Dust Concentrations and Psychrometry

Run Number	Brick Treatment	Impinger Counts (M.P.P.C.F)*					Psychrometry (deg. F)					
		1st Pair	2nd Pair	Averages			Before test			After test		
				1st Pair	2nd Pair	All	w.b.	d.b.	RH%	w.b.	d.b.	RH%
1	None	31.4 29.6	23.1 21.2	30.5	22.2	26.3	69.0	75.0	74	77.3	79.5	91
2	1.0 pound per gallon	2.1 2.8	3.9 4.2	2.5	4.1	3.3	68.0	71.7	83	73.0	75.2	90
3	Water only	11.7 12.0	13.1 12.8	11.9	13.0	12.4	68.9	74.0	77	75.0	77.5	89
4	0.5 pound per gallon	2.9 2.9	3.8 4.2	2.9	4.0	3.5	63.0	77.0	46	78.0	79.8	93
5	0.25 pound per gallon	3.8 3.1	5.1 4.6	3.5	4.9	4.2	60.3	71.3	53	73.2	75.5	90

*Million particles per cubic foot of air.

operations with treated and with untreated bricks. The reduction resulting from treating the bricks with water is about 50%. An additional 70% reduction results from the presence of 0.5 pound per gallon of ammonium lignin sulfonate in the water. The total reduction from untreated to treated bricks is therefore about 85%.

Possibly a better comparison for practical purposes would be that between the first pair of samples taken in each run, since this reflects the first few handlings of each brick, the condition most likely to be found in actual laying-up practice. Using these data, the total reduction is about 90%. In either comparison the reduction of dust dissemination is very significant considering the highly silicious character of this type of refractory brick.

It is of interest that run number one in which the bricks were untreated differed from all other runs in two consistent respects. The first run was the only one in which the first half hour of brick manipulation produced higher dust counts than the second half hour. At the same time, the mean particle size from this test was appreciably higher than from any other. A reasonable explanation of these facts is that some of the dust dispersed from untreated bricks is loose on their surface and is disseminated by handling. Such dust was visible when the pallet was broken open. While some of this loose dust may be removed by water dipping, a much larger proportional reduction results from the surface treatment of the bricks with ammonium lignin sulfonate. This treatment is presumed to be effective in dust reduction, not only by washing off loose dust but additionally by providing a light bonding which either prevents dust dispersion from abraded surfaces or causes particle agglomeration to non-airborne sizes.

There was no critical concentration of the treating chemical discovered within the series studied. However it is notable that there was no significant improvement from the twelve per cent concentration compared with the six per cent concentration. The twelve per cent solution that Cline and Bloom[6] described as being effective in actual furnace construction operation can probably be halved without sacrificing the dust control benefits.

Summary

Silica brick treated with a solution of ammonium lignin sulfonate to a penetration of about one-half inch produced less air-borne dust than untreated brick or than brick treated with water only. The reduction in dust concentration was of significant magnitude, being about eighty-five to ninety per cent when comparing the results of optimum treatment with untreated brick and about seventy per cent when comparing results of optimum treatment with water-dipped brick. The optimum concentration was 0.5 pound or more per gallon of water (about six per cent or more by weight).

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Hygienic Guides and Binders Available

INDIVIDUAL Hygienic Guides in loose-leaf form may be obtained from the American Industrial Hygiene Association, 14125 Prevost, Detroit 27, Michigan, at 25¢ each. Discount of 20% allowed on orders of five or more; 40% on orders of 100 or more. Also available are flexible loose-leaf binders for the individual Hygienic Guide sheets. The binders have been especially designed to provide maximum protection and ease of handling of the Guides. They will be particularly useful in keeping a permanent file. The binders are blue in color, with white lettering, and are fitted with ¾″ rings. The price is $1.25. The Guides and the binders may be ordered on the blank on page 264.

A Portable Multi-Range NO_2 Gas Monitor*

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A portable monitor for recording concentrations of NO_2 during environmental and stack sampling is described. The sample flows concurrently with the reagent, N-1 naphthylethylenediamine dihydrochloride, through a beaded column. Diazotization of the reagent produces a colored solution which is measured by a flow-through colorimeter. Concentrations ranging from 0.5 ppm to 15,000 ppm can be measured.

Introduction

TWO TYPES of samples that are normally employed for determination of the concentration of NO_2 are grab and integrated samples. These samples are usually adequate if taken in sufficient number to be representative, but they often fail to reveal with sufficient accuracy the peaks and fluctuations in concentration. Also, during stack gas sampling, process operations frequently have to be followed continuously to demonstrate the desired correlation between emission rate of this gas and process variables.

To provide the type of information discussed above, a prototype NO_2 monitor incorporating basic design features reported by Stanford Research Institute[1] was constructed. Some of the design criteria were portability, a response time to an NO_2 cloud of less than one minute, ability to record NO_2 concentrations from 0.1 ppm up to 5,000 ppm with provision for extending the range to 15,000 ppm, and ability to operate unattended for one week. Because various oxides of nitrogen frequently occur together it was desired to be able to measure NO_2 and NO separately; however, provision was not incorporated to permit this differentiation at this time.

Experimental Apparatus

Design Layout

The major components of the instrument, as shown in Figure 1, were a constant-head vessel for the reagent, a reaction column, a spectrophotometer, an A. C. amplifier and a recorder. The reagent flowed from the constant head vessel through a small screw clamp and rotameter into the reaction column as shown in Figure 1. The reagent flow rate into the column was maintained within plus or minus three per cent at the lowest rate. The constant head vessel was equipped with a pressure equalizing system that makes it possible to sample from atmospheres having pressures fluctuating considerably above or below atmospheric pressure with no effect on reagent flow rates.

The gas sampling rate of the apparatus can be varied from 5 to 400 cc/min. The air flow into the column was regulated by a needle valve and measured by a rotameter. When the monitor was used with dilution air, for elevated concentrations, a combination of two rotameters was used, one for dilution and the other for sample.

The dimensions of the column were critical and an optimum absorption time was achieved with a column diameter of 5.7 mm ID and a length of 6 inches. With a larger bore, the liquid did not give uniform wetting and tended to channel. Three millimeter glass beads were most satisfactory.

The sample and reagent are brought together at the top of the column and flow concurrently over the beads. After the reaction, the color density is measured in a flow-through light absorption cell (540 mu) and then the reagent is collected in the reagent waste container. Light that penetrates the developed reagent is detected on a phototube and the resulting current is amplified and recorded. The light absorption cell was designed with minimum dimensions to permit the entire light beam to pass through the solution and to facilitate rapid flow of the reagent. It was found that a cell having a ⅝ inch inside diameter and a ¾ inch overall length including sharply tapered ends was best suited for this particular instrument. Smaller sizes did not make full use of the light path whereas any larger size would not exhibit changes in concentration with sufficient rapidity. A square corex (10 mm) cell with highly polished windows was tried and gave good sensitivity, but time lapse took approximately 30 minutes for the

* Work performed under Contract No. W-31-109-Eng-52 between the Atomic Energy Commission and General Electric Company.

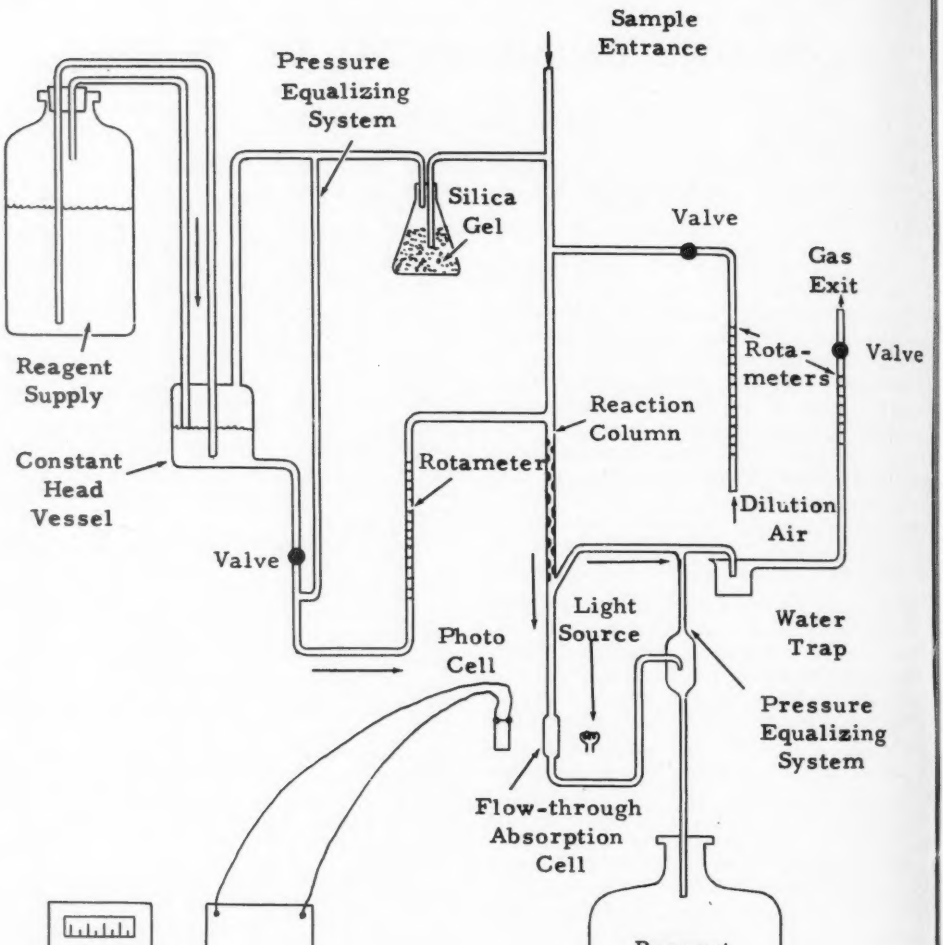

FIGURE 1

instrument to record an equilibrium point. The equilibrium point was reached at the time of maximum color development for the instruments system. This time was usually 5.7–7 minutes, depending on which reagent* was used. Figures 2 and 3 show the time lapse for these two reagents, and the instruments' response times to NO_2.

* See APPENDIX.

ELECTRONICS

A Coleman Universal Spectrophotometer, Model 11, was modified to measure the light absorption of the reagent. The resulting phototube

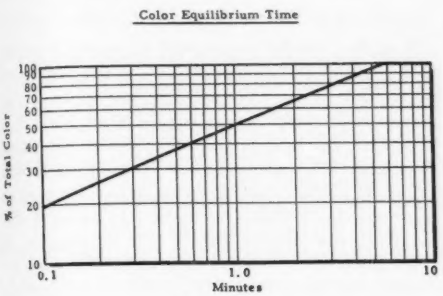

FIGURE 2

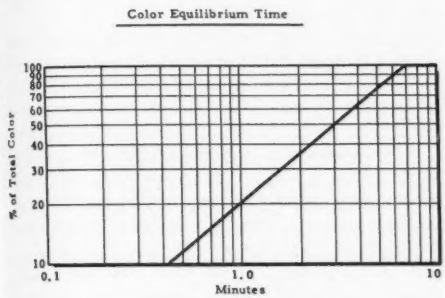

FIGURE 3

current of the spectrophotometer was fed into a Millivac Micro-Micro Amperemeter, Model BX, which amplified and helped stabilize the current recorded on an Esterline-Angus Recorder, 0.0–1.0 milliampere.

A voltage regulator was placed at the power source to maintain 110 volts to the apparatus. Any variation in the voltage to the exciter lamp markedly affected the light output.

REAGENTS

The two reagents used in the instrument are those reported by Saltzman[2] (reagent A*) and by Brodey and Jacobs[3] (reagent B*). These two reagents are useful for different applications of the monitor, the final selection being determined by the type of measurements desired. Reagent A is used in conditions where the concentration is relatively low and constant and a quick response to the gas is not needed. Reagent B is used for environmental sampling where a quick initial response is needed.

Calibration of the Instrument

PREPARATION OF TEST ATMOSPHERES

The calibrations were made from known concentrations of NO_2 that were drawn from 10 or 46 liter carboys as shown in Figure 4. These carboys were evacuated to a few mm of Hg and the NO_2 was introduced by a syringe or from a weighed $Pb(NO_3)_2$ crystal which was decomposed in a heated tube. In either case, the NO_2 was drawn into the carboy by releasing the vacuum. When introduced from a syringe, the NO_2 was made from NO. This was done by forcing NO into a syringe from a compressed gas cylinder. Atmospheric O_2 was then added to the NO, which reacted to form NO_2. NO_2 formed in this manner still had a small amount of NO present. All known concentrations of the NO_2 gas were verified by sampling with a syringe or an evacuated flask. The concentrations were determined by the phenol-disulphonic acid method[4] using evacuated flasks, and by reaction with reagent A or B by standard procedure for syringe samples. The standard procedure for the syringe method consisted of drawing a sample and then allowing it to react with the reagent for a 20-minute period, followed by reading on a spectrophotometer.

The air drawn from the carboys during calibration tests was replaced by air which flowed into the plastic bag contained in the carboy, as seen in Figure 4. This technique prevented dilution of the test atmosphere during sampling.

During calibration of the instrument involving a series of tests from the same source of known concentrations, the light transmission was observed to increase gradually. The concentrations of the chamber were checked before and after each calibration test was made, and found to decrease 5 per cent over a period of one hour. This was due to adsorption of NO_2 on the walls of the carboy and the plastic bag.

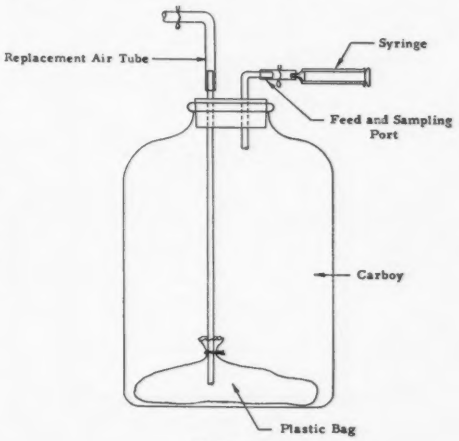

FIGURE 4

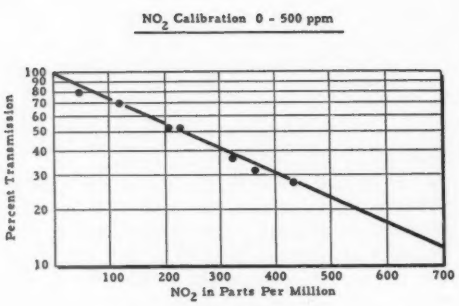

FIGURE 5

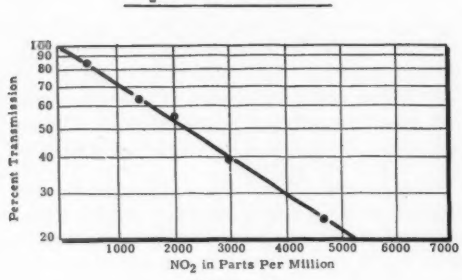

FIGURE 6

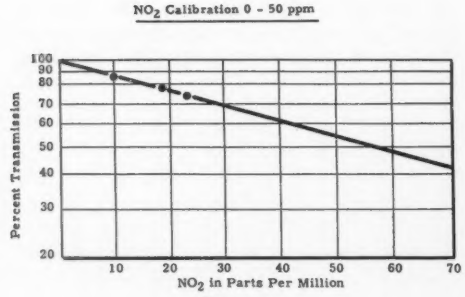

FIGURE 7

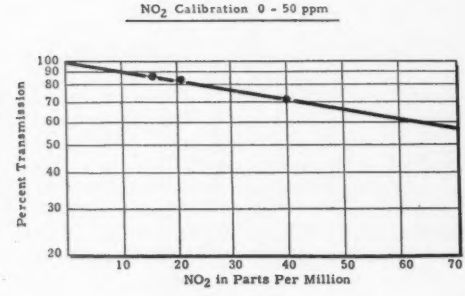

FIGURE 8

CALIBRATIONS WITH VARIOUS CONCENTRATIONS

Selection of the reagent for calibration of the instrument to NO_2 clouds was dependent primarily on the concentrations involved and the necessary rapidity of response. For these reasons the instrument was calibrated in three ranges of NO_2 concentrations; 0–50 ppm, 0–500 ppm and 0–5000 ppm. Two techniques were used to attain the 0–500 and 0–5000 ranges for the monitor; first, by dilution (1:1) of reagent B with distilled H_2O and, secondly, by supplemental dilution (10:1) of the NO_2 sample with clean air. Variation in the sampling rate would make higher determinations of about 15,000 ppm possible.

Figure 5 shows the results of the calibration of 0–500 ppm NO_2. For each point on the graph, the concentration of NO_2 was checked before and after the calibration sample was taken. The carboy was connected to the sampling port of the monitor and the instrument was run until the equilibrium point or maximum color development was reached. These points were plotted as a function of percent light transmission and concentration to give the calibration. A lower limit of 10 ppm was measurable for this range. The flow rates for air sample and reagent were 57 cc/minute and 6 ml/minute.

The calibration shown in Figure 6 was similar to tests shown in Figure 5. The air in this test was diluted (1:10), and the instrument was calibrated between 0–5000 ppm NO_2.

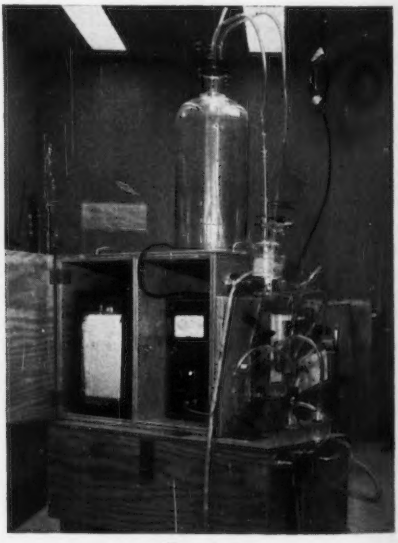

FIGURE 9

The results of the calibrations of 0–50 ppm range with Reagent A and Reagent B are seen in Figures 7 and 8. Reagent B was used for low (1–10 ppm) concentrations of NO_2 that persisted for less than one minute, whereas Reagent A was used for low concentrations that persisted for longer periods of time. The flow rates for air sample and reagent were 400 cc/minute and 1.5 ml/minute.

Performance

After completion of laboratory tests, the components were fitted into two carrying cases, as shown in Figure 9, to facilitate transportation of the monitor. In actual use, the monitor exhibited a background drift that was due to heat and degradation of the lamp. The A.C. amplifier was cooled by a 1 cfm air blower which eliminated most of the drift. Variations in line voltage were held constant by a Sola voltage transformer. These variations in the line voltage were caused by the pumps on the same electrical line. The reagents were sensitive to the direct rays of the sun and needed to be shielded.

Summary

A prototype portable NO_2 monitor has been developed which has the following features. A lower limit of 0.5 ppm NO_2 can be detected. The response time of the instrument is less than 1 minute to a contaminated atmosphere. The maximum level is approximately 15,000 ppm NO_2. Sampling is possible at positive and negative pressures by a regulating system. The instrument can be operated for a period of one week with a minimum amount of attention.

References

1. Private communication (W. D. Thomas, Stanford Research Institute).
2. Saltzman, B. E., Colorimetric Microdetermination of Nitrogen Dioxide in the Atmosphere, *Analytical Chemistry 26:* 1949 (Dec. 10, 1954).
3. Jacobs, M. B. *The Analytical Chemistry of Industrial Poisons, Hazards and Solvents,* 2nd Edition, p. 357, Interscience Publishers, Inc., New York, 1949.
4. Beatty, Berger and Schrenk, *Determination of the Oxides of Nitrogen by the Phenoldisulphonic Acid Method,* Bureau of Mines, R. I. 3687, Feb. 1943.

Appendix

Reagent A—140 ml of acetic acid
5 grams of sulfanilic acid
20 mls of 1% N - 1 naphthylethylenediamine dihydrochloride
diluted to 1 liter

Reagent B—0.1 gram N - 1 naphthylethylenediamine dihydrochloride
0.4 gram of sulfanilamide
9.5 grams of tartaric acid
diluted to 1 liter

Gas Chromatography in Industrial Hygiene and Air Pollution Problems

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Foreword

WITHIN the last few years the technique of gas chromatography has grown very rapidly. Most recent publications on the subject have been concerned with applications to analyses performed in the petroleum and fuel industries. That a technique in its infancy should be applicable to complex mixtures of hydrocarbons points up its remarkable power in analytical work. Little work has been published on trace analyses as the term is understood by workers in the fields of industrial hygiene and air pollution. One purpose of this paper is to examine the technique in the light of the special requirements of these two fields. Another purpose of the paper is to familiarize workers in these fields in a general way with the principles and apparatus used. For historical, constructional, and operational details the reader is referred to the bibliography. The review by Phillips[1] is recommended for a comprehensive summary of information on the subject.

Parts of this paper are largely speculative. There is considerable justification for this because sufficient work has been done to serve as a basis for such conjecture and the fundamental process is fairly well understood. Chemists often predict that two liquids can be separated by distillation even though there is no experimental evidence that such a separation is possible. The strong analogy beween distillation and the gas chromatographic method permits prediction with considerable confidence. The factor of reasonable predictability is one of the main assets of the method.

For the sake of definiteness, the writer often has utilized examples from his own experience. In such instances, no experimental details have been given, since the paper is intended only to present the possible scope of gas chromatography.

Presented at the A.I.H.A. meeting in St. Louis, Mo., April 25, 1957.

Basic Principles and Apparatus

The separations of gases and vapors which are obtained by a gas chromatography column are due to variations in the affinities of the gases for the material with which the column is packed. (Workers in the industrial hygiene field are familiar with this principle of varying affinity through their experience with sampling vapors by adsorption on silica gel or charcoal.)

Suppose that a long small tube is packed with silica gel and that a (carrier) gas is passed through the tube at a constant rate. Eventually, the gases (O_2, N_2, etc.) which are normally adsorbed in silical gel will be purged by the carrier gas and a state of equilibrium between the gel and the carrier gas will be attained. Now suppose that a mixture of two vapors—benzene and pentane—is introduced into the gas stream at the inlet of the tube. Momentarily, both of these vapors will be completely adsorbed near the inlet of the tube. However, because of its lesser affinity for silica gel, the pentane will be desorbed more easily than the benzene and will be carried a little further by the carrier gas. This process of adsorption and desorption will be repeated many times in the tube and each time the pentane will move even further ahead of the benzene. If the tube is long enough, the two substances will be completely separated when they reach the exit of the tube. When a suitable detector and recorder system is attached at the exit, a peak corresponding to each substance will be observed on the recorder chart. (See Figure 2.) These peaks are frequently of the form

$$y = ae^{-bx^2}.$$

By increasing the column length the peaks can be separated even more. The same effect can be accomplished by using a more active column packing such as charcoal. Temperature, gas flow rate, and other factors also affect the sharpness of separations and peak shapes.

In order to obtain consistent results, it is necessary to control the various operating pa-

rameters rather closely. Most of the equipment is designed to accomplish this control and its relative complexity is due chiefly to regulators of various types.

The basic elements needed in a usable gas chromatography apparatus are shown schematically in Figure 1. The carrier gas, usually helium, flows through the column at a constant controlled rate. The sample of gas or vaporizable liquid is introduced into the carrier gas near the inlet of the column. The exit gases from the column and the pure carrier gas are continuously compared by a detector, the output of which is fed into a suitable recorder. The column is made usually of ¼-inch copper, glass, or stainless steel coiled tubing and may be from one to a hundred feet long. The nature of the column packing will depend upon the separations which are desired. A constant temperature bath is provided for the column.

The detector is ordinarily a differential thermal conductivity (T.C.) cell which forms a part of a Wheatstone bridge circuit. More specialized detectors have been used.[1, 2] Some are more sensitive or more specific, but the T.C. type has proved most generally useful. The electrical output of the T.C. cell is measured by a recorder which should have a range in the order of a few millivolts.

The sample can be introduced into the carrier gas stream by means of a hypodermic syringe. More elaborate and accurate sample injection systems are available on commerical instruments or can be constructed.[3]

There are three important gas chromatographic techniques: (1) adsorption-elution chromatography, (2) gas-liquid partition chromatography, and (3) adsorption-displacement chromatography. The adsorption-elution technique has been illustrated in the second paragraph of this section.

If the column is packed with an inert support (*e.g.*, ground firebrick) coated with a high-boiling liquid such as di-n-butyl phthalate or Silicone® oil, then one can use the technique of gas-liquid partition chromatography. The bases for the separations in this instance are the differing affinities of the sample components for the coating on the inert support. Such affinities are primarily functions of solubilities of the gases in the liquid coating. This type of chromatography is especially useful in solvent analysis. Peaks are usually sharper, better shaped, and better separated than with the adsorption-elution technique.[1] The gas-liquid partition method is perferred for general applications.

In adsorption-displacement chromatography the sample is adsorbed on the column and then is driven off by introducing a displacing vapor

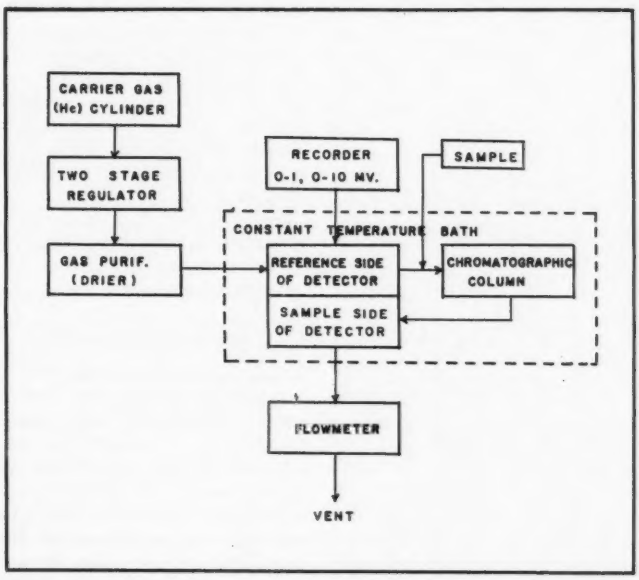

FIGURE 1. Schematic Diagram of a Simple Gas Chromatography Apparatus

into the carrier gas. This displacer acts by virtue of its greater affinity for the adsorbent and by the mass action principle. The sample components are detected as they emerge from the column. This technique has been utilized extensively in gas and solvent analysis and appears to the writer to hold great promise for the concentration of trace components.[4] Obviously, this method involves more parameters which must be controlled than do the other two methods.

Adsorption-elution and liquid-partition methods require exactly the same apparatus except that different column packings are used. The adsorption-displacement technique requires a means of introducing the displacer in the carrier gas. This is most easily accomplished by bubbling the carrier gas through the liquid form of the displacer. The liquid is kept at constant temperature in order to secure a constant concentration.

With any of the methods, it is apparent that all operating conditions must be controlled in order to obtain reproducible results. Under a given set of parameters, the time required for elution of a certain substance is a property of that substance. This is known as the elution or retention time. It is almost independent of the amount present. The retention time multiplied by the gas flow rate gives the volume of carrier gas required to drive the substance from the column. This is called the retention volume. It is useful when comparisons are made of the performances of similar columns operating with different flow rates and/or with different lengths. Compounds are identified by their retention times; the areas under the respective peaks are related to the quantities of the compounds.

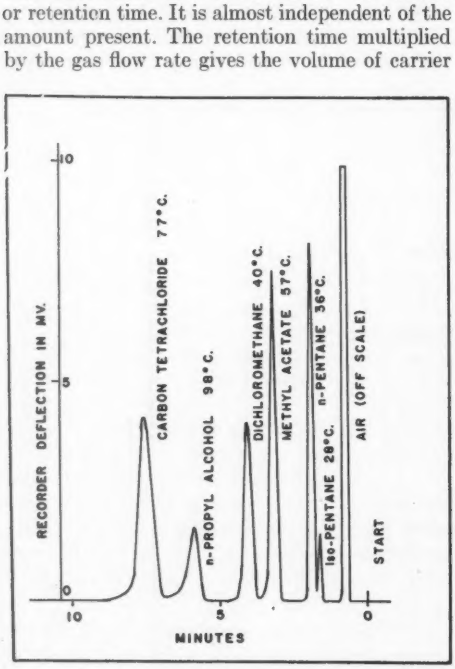

FIGURE 2. A Typical Gas-Liquid Partition Chromatogram of an Easily Resolved Mixture.

Sample sizes vary with circumstances but run around 0.05 to 25 ml for gases and 0.001 to 0.25 ml for liquids. Naturally, the sample size will be determined partly by the sensitivity of the instrument. This in turn depends upon operating parameters and especially upon the carrier gas used. The nature, number, and relative amounts of the components of a mixture have a bearing on the sample size. This is true of gases and liquids. Where only one or two foreign components are present and desired in air, a large sample (50–100 ml) sometimes can be taken without prior concentration of the contaminants.[5]

Figure 2 shows a typical well-resolved gas-liquid partition chromatogram obtained by the writer for illustrative purposes. The constituents were selected purposely for good separations. The sample was one milliliter of air which was taken from a bottle containing the liquid mixture. (The substance marked "iso-pentane" was present as an impurity in technical grade pentane and its identification is subject to some question. This instance illustrates the type of information which often can be obtained without additional effort.) The chromatogram indicates that the column packing has a relatively greater affinity for halogenated hydrocarbons than for alcohols and esters. It will be noted that the boiling point is not the only factor affecting elution time. However, with closely related compounds, it is generally the controlling factor. Obviously, gas chromatography is not a distillation process but it is analogous to distillation. It seems to the writer that the gas chromatographic process is the more general process.

Early Applications in Industrial Hygiene

Adsorption chromatography in a crude form can be traced back to the early part of the century.[6] Its principles have been applied for a number of years in atmospheric sampling. In his book, Elkins[7] describes a method for separating certain halogenated hydrocarbons. This method is based on adsorption followed by aeration (elution) with controlled volumes of air. According to the same source benzene and toluene can also be separated in a similar way. Peterson, Hoyle, and Schneider[8] reported on a

like method in 1956. These techniques qualify as gas chromatography even though they were not called such by the writers. Both of these groups of experimenters clearly recognized the principle of the constancy of the retention volume. Thus it will be seen that the principles have been quite familiar to industrial hygiene workers for some time. The concept of retention volume is an exceedingly important one in atmospheric sampling.

James and Martin[9] in 1952 originated gas-liquid partition chromatography which is perhaps the greatest advance in the field. This technique permits one to operate at lower temperatures, to obtain better separations, and to use a wider variety of column packings than was previously feasible. These attributes permitted the extension of the process to include liquids with relatively high boiling points.

Present Applications

The field is expanding at such a rapid pace that it is difficult to state just what the present status is. A considerable amount of specialized work has been done.[1, 4, 10] In this section the writer is limiting his discussion generally to those things which have been done and which could be repeated on commercially available equipment.

Applications of the Thermal Conductivity Cell

Differential detectors, particularly the thermal conductivity type, should find more applications in laboratory setups and in toxicological exposure chambers than they have in the past. Within the last year or two considerable advances have been made in increasing the sensitivity of these devices. Such cells can be used to monitor gas streams continuously and could be made to maintain essentially constant concentrations of vapors in such setups.

Application to Solvent Analysis

Solvent analysis is one of the most difficult problems with which the industrial hygiene chemist is faced. Such analyses are tedious and the results are usually subject to considerable question both qualitatively and quantitatively. The most obvious application of gas chromatography to industrial hygiene is in this area.

The analysis of a solvent is performed usually in order that the qualitative and quantitative determinations of workers' exposures can be done. This is done in most cases by analyzing the air for the most toxic substances present. Thus the solvent analysis itself is often only a means to an end. Present gas chromatographic techniques do not have the sensitivity to determine solvents in air at most threshold concentrations. Nevertheless, the concentrated vapors of a solvent (from a stoppered bottle or from a tank) can be determined. Such an analysis often eliminates the necessity for the actual solvent analysis and provides more directly the information usually desired.

It would appear that eventually direct air analysis will be possible and essentially all vapors present will be determined in one sample. Furthermore, this will be done with less labor than is now required for the determination of one constituent by ordinary chemical methods.

There is a sour note in solvent analysis. The number of possible compounds rapidly increases with increasing boiling point. This means that overlapping or superimposition of peaks will occur. Some preliminary separation *(e.g.,* by fractional distillation) likely will be desirable, even if for no purpose other than to prevent fouling the column with high boiling substances. Solvents are more easily handled if all components have boiling points within a fairly restricted range.

The Determination of Minor Constituents

Occasionally, one wishes to determine a minor or trace consituent in a pure compound or a mixture of compounds. For example, the writer's first application of gas chromatography was to the determination of traces of benzene in paint thinners. If the major constituents give peaks which are well-separated from the trace peak, then the problem can be solved at least to the extent of establishing a maximum figure for the trace or minor component.

The writer had occasion to check supposedly pure ortho-, meta- and para-xylene by gas chromatography. Two isomers showed traces of lower aromatics while the third isomer showed all isomers and lower aromatics in relatively large amounts.

This technique should help in the analysis of solvents for additives of various kinds.

Ordinary Gas Analysis

Gas analyses such as are ordinarily done with an Orsat apparatus can be easily and quickly done with the presently available equipment.[1, 11] The accuracy compares favorably with the conventional methods for the major components and the minor components can be determined with greater accuracy unless a specially designed burette is used with the Orsat apparatus. Analyses of this type are very quickly done and no

attention is required after the sample is injected. An air analysis for oxygen and nitrogen can be done in five to ten minutes if a suitable column is used.[5, 11]

Potential Applications

The applications discussed in this section are those which are fairly obvious but which have not been reported in the literature at the time of this writing. Some of these processes have been carried out at high concentrations or under specialized conditions. The transition to required conditions should be possible, but will require considerable study in many of the cases cited.

The Column as a Separating Device

The really difficult problems of analytical chemistry usually lie in the field of separations. It is the extreme separating powers of the chromatographic column which make the technique such a revolutionary one. There are many problems in air analysis which require a means of separating gases or vapors from one another. Often a sensitive group reaction is available, but is useless because other members of the group are present and preclude its use. The difficulty may be that interfering members of another group are present. Since a compound is eluted at a given time, it can be separated from all compounds not having the same elution time. Even for compounds having similar elution times, the case is far from hopeless because often these compounds may be separated by passing them through a column with a different packing.[12]

The technique lends itself readily to the introduction of reacting columns or purification trains. As an illustration, consider the separation of alcohols, esters, and ketones from saturated aliphatic hydrocarbons. This separation can be accomplished easily by the insertion of a short column packed with sand, crushed glass, or other inert support coated with 95% sulfuric acid.[12] Likewise carbon dioxide, water, hydrogen sulfide, etc. can be removed by conventional reagents packed in short tubes. These additional columns will change the elution times somewhat and will have to be present when such elution times are determined.

It should be pointed out that the sample size is quite small and that very sensitive analytical methods are needed to detect the separated substances. Another approach is to increase the size of the column in order to accommodate a larger sample. Large-capacity columns are available and are used commercially to produce especially pure compounds.[13]

Separation and Determination of Group Members when Present Together

The word group is used here in the sense that its members share a common property which is also the basis for the determination of the members when present alone. This definition will be seen to cover rather dissimilar substances as well as many of the common organic groups such as aldehydes, alcohols, ketones, and the like.

As an important example consider a mixture of aldehydes in air: formaldehyde, acetaldehyde, and acrolein. Any one of these, if present alone, could be determined by Schiff's reagent. Suppose a fourth aldehyde was suspected to be present along with numerous other compounds; how could one definitely determine the three compounds and also detect the fourth, if present? Gas chromatography offers a simple solution. The fractions coming off at the appropriate times simply are separated and tested with Schiff's reagent for the three known aldehydes. The remainder of the eluted compounds are combined and tested for aldehydes. A positive test would indicate that a fourth aldehyde was present. (It is assumed that Schiff's test is sufficiently sensitive.)

Again consider the compounds present or formed when halogenated hydrocarbons are burned. What are they and what are their relative amounts? Many of the substances present contain chlorine and, therefore, a simple chlorine analysis would be of little value. Phosgene, hydrochloric acid, chlorine, and the halogenated hydrocarbon itself have been identified or suggested as being present. The analysis of such a mixture is quite difficult and dubious. Gas chromatography offers a possible simple solution to this problem and to many others like it. In applying it, one is likely to find compounds not previously recognized as being present.

Air Analysis for Traces of Contaminants

Under the usual conditions of operation a commercially available apparatus had to be pushed to determine carbon dioxide in normal air (0.03% or 300 ppm) on a 25-ml sample without preliminary concentration.[5] Under different operating conditions, carbon monoxide was easily detected and determined in air when present to the extent of 800 ppm.[5] It was estimated that a concentration of 100 ppm could have been determined with certainty. Thus it appears that substances having threshold limits of 1,000 to 100 ppm likely could be determined on a 25-ml air sample. Yet the range of 0.01 to 100 ppm is of interest to those interested in air pollution and industrial hygiene.

The problem then is to concentrate the contaminant sought, to devise extremely sensitive detectors, or to do both. Certain detectors are sensitive to a few ppm of certain substances, but have other serious drawbacks.[1, 2, 4, 14] T.C. detectors have been made more sensitive, but it is doubtful that this type can be improved in sensitivity by a large factor. The more promising approach appears to lie in the concentration of the compounds sought. Conventional methods are (1) freeze-out techniques, (2) adsorption on silica gel or charcoal, (3) solution in a suitable inert solvent, (4) absorption in a chemically reacting solution, and (5) methods used for particulates when the substances have a very low vapor pressure.[7] All of these could conceivably be worked into a gas chromatographic method, but method (2) appears to the writer to be by far the most promising because it is already gas chromatographic in nature.

The problem fundamentally is this: one needs to transfer a sufficiently large sample of the contaminants to the column within a period of time which is short relative to the elution times of the compounds sought and, at times, air needs to be removed. At the worst, one should be able to bring the sample onto the column in a reproducible way.

There are two ways by which this might be done. The first of these is by applying properly controlled heat to a closed vessel containing the adsorbed sample on silica gel or charcoal. After thermal equilibrium is established, the desorbed gases can be flushed out and onto the column.

The second method of transferring would make use of the adsorption-displacement technique. The apparatus would consist, in effect, of two columns. The usual analyzing column would be preceded by a sampling column containing the adsorbent. (The latter column might be used in the field as a sampling device or the adsorbent only might be transferred to the column in the laboratory.) In this method the contaminants would be removed from the adsorbent by introducing a displacer (such as bromobenzene) into the carrier gas. A pilot detector would need to be inserted between the two columns in order to determine the "break through" of the displacer from the sampling adsorbent. The analyzing technique could be by the gas-liquid partition or by adsorption-elution techniques. To the writer this procedure appears more practical than the thermal one mentioned above. Such a process involves additional parameters which must be controlled.

The writer would like to point out that this latter technique has been used extensively as an analyzing technique with relatively high concentrations of compounds. Fortunately, going to lower concentrations does not affect the chromatographic process noticeably and the procedure should work.

The determination of hydrocarbons in air by a gas chromatographic technique involving concentration was announced by the Shell Development Co.[15] A four-liter air sample was used and hydrocarbons were determined in the range of 0.02 to 1.5 ppm by weight. The hydrocarbons were trapped on a short gas chromatographic column immersed in liquid oxygen. They were removed by warming and were introduced into a conventional analyzing column. The method is analogous to the thermal desorption method outlined above. This work indicates that low concentrations of substances are determinable. However, not all substances would be expected to yield to the relatively simple technique employed. The nature of the sample is exceedingly important in the selection of concentration methods and of column packings.

Applications to Sampling Techniques

There are numerous questions about sampling by adsorption which arise in practice and which previously have been answered in an empirical way if answered at all. The technique offers an easy method for analyzing for almost any vapor and thus makes detailed studies of sampling procedures feasible. A more far-reaching contribution is that the theoretical advances in the technique give a basis for organizing previously unconnected fragments of information and have enabled us to understand adsorption phenomenon better.

For example, the concept of retention volume will enable one to predict the maximum air volume which can be sampled quantitatively with a given adsorbent under a particular set of circumstances. Heretofore, a careful worker reproduced the sampling conditions of the original investigator or checked his own apparatus for its efficiency. Along the same lines, the technique will enable one to predict the relative affinities of various compounds for adsorbents. Of course, this will be done experimentally, but it is so easy to do that the information should be forthcoming. A new insight will also be provided to the behavior of mixed vapors in adsorption phenomena. With more information of this type available, adsorption methods should enjoy a renewed popularity.

It is evident now that all gases are theoretically adsorbed quantitatively on silica gel and charcoal. Any inefficient collection in sampling air is due to desorption of the substance and elution

by the air sample itself. Thus, one can collect hydrogen on silica gel, but a very small air sample must be taken. In practice, this may amount to no adsorption.

Evidently, sampling methods in general can be more easily studied because of the analytical simplicity of the technique.

Applications to Biological Samples

Many substances are present in biological samples to the extent that they could be determined by present equipment. However, suitable methods of isolation would need to be employed. For example, benzene could be determined in urine by a distillation and extraction procedure if a suitable extractant (pentane) be used. Other volatile extractable compounds would not interfere unless they had essentially the same elution time as benzene.

Many volatile metabolites of organic substances should be determinable and identifiable by the technique.

Change of Gaseous Phase

There are a few cases in which it is desirable to change the gaseous environment in which a substance exists. For example, if a chemical test is sensitive to oxygen, it is necessary to perform the test in an inert atmosphere. In most counting techniques, radon needs to be counted in an oxygen-free gas. Gas chromatography provides a means for easily accomplishing such changes in gaseous environment.

Limitations

The technique has some drawbacks. The first of these is its lack of sensitivity. This is partly because of the small sample which is used. (Sometimes this requirement is an advantage.)

Because of the small sample size, very delicate chemical tests are needed for detecting eluted compounds. Chemists will undoubtedly be faced with this problem during the next few years.

The technique measures a property which is not unique and thus it may be that other substances are responsible for part or all of the observed effect. A great deal can be accomplished to overcome this by varying columns, operating parameters, and types of detectors.

Reactions may take place in the concentration of the sample, on the column, or in the detector. Decomposition in the detector does not necessarily bar the method.

Equipment Requirements and Cost

The two most important requirements on equipment for industrial hygiene work are its sensitivity and its versatility. The latter can be judged rather easily from the manufacturers' brochures but the information on sensitivity it often vague or difficult of translation in terms of the worker's needs. An expression for detector sensitivity has been given.[3] A constant temperature heating chamber is extremely desirable. Most models cost from $2500 to $3000 complete with a recorder.

Conclusions

Some of the fundamental principles of gas chromatography have been utilized in the fields of industrial hygiene and air pollution for many years. In its present status, the technique and available equipment offer solutions to difficult and time-consuming analytical problems such as solvent analysis and ordinary gas analysis. Generally speaking, commercially available equipment does not have the sensitivity required for the direct analysis of air for trace constituents, but certain components present to the extent of about a hundred parts per million can be determined directly. The lower limit on sensitivity cannot be definitely stated because it depends upon too many factors including the type of sample. Some known methods for concentrating samples have been discussed in relation to the gas chromatographic technique.

The technique, if applicable to a sample, yields accurate, quick, and reproducible results with the expenditure of very little labor and time.

The theory and practice of gas chromatography should provide a large amount of information on sampling and analytical methods used in industrial hygiene and air pollution work.

Many problems remain to be solved, but the field is extremely promising.

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Upper Limits of Thermal Stress for Workmen

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Introduction

OBSERVATIONS by Haldane[17, 18] were the earliest that described with reasonable accuracy the tolerable upper limits of thermal stress for workmen. The velocity of circulating air and the temperature of the wet-bulb thermometer were, according to Haldane, the factors of greatest importance to men working under severe heat stress. He concluded that the upper limits of thermal stress that could be tolerated safely by workmen were 88°F. wet-bulb in still air and 93°F. wet-bulb in circulating air having a linear velocity of 150 feet per minute. Sayers[20] concluded that the upper safe limit among miners was in the neighborhood of 90° to 93°F. wet-bulb temperature. In this connection dry-bulb/wet-bulb temperatures were not reported at the specific locations in which collapse of miners had occurred but it was stated that workmen experienced nausea and headache when the temperatures were in the range of 90°/90°F. (dry-bulb/wet-bulb) and 100°F. dry-bulb with 89 per cent relative humidity.

During the intervening years there have been a large number of investigations made under carefully controlled conditions of temperature and humidity and in which extensive observations were made on human experimental subjects.[1, 2, 3, 6, 7, 10, 11, 12, 13, 14, 15, 19, 20, 22, 27, 28, 29, 30] Much effort has been expended on measuring the effects of thermal stress on man in relation to (a) the degree of acclimatization, (b) rates of work and metabolic generation of heat, (c) type and amounts of clothing worn, (d) gain or loss of heat due to radiation and/or convection, (e) cardiovascular responses, (f) rates of sweating and the chemical composition of perspiration. Although much effort has also been made to relate loss in productivity to increasing thermal stress on workmen, the degree of success has not been consistent due to difficulties relating to interpretation of the variations in motivation of the persons being tested. Heat exhaustion or collapse remains the one physiological response or end point that can be predicted reasonably well. Certain already published data can profitably be plotted together on graphs with a view to appraising certain suggested upper limits of thermal stress for man.

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The Data

At Ft. Knox, Kentucky, three curves were fitted to data obtained in relation to fully acclimatized volunteers of the armed forces who were subjected to high levels of thermal stress.[12, 13] In terms of increasing severity of the climatic conditions tested, these curves were labelled "RELATIVELY" EASY, DIFFICULT, and IMPOSSIBLE, see Figure 1. Similar observations made in Europe[6, 7], Africa[10, 11, 19, 20, 28, 29, 30, 31] and Asia[1, 2, 3, 14, 15, 22] yielded data that corroborate fairly well the data obtained at Ft. Knox.

Men were acclimatized experimentally by Weiner[27, 28] who exposed the men to dry-bulb temperatures in the range of 96° to 98°F. and wet-bulb temperatures in the range of 94° to 95°F. In a group of 36 persons who were being acclimatized at these temperatures, 6 collapsed. These cases of collapse, if plotted on the accompanying graphs, see Figures 1 and 2, would be located near the line labelled DIFFICULT. It may be noted here that among properly acclimatized subjects at Ft. Knox, no cases of collapse were reported at similar levels of thermal stress.

Particular reference is made to the data recorded in Report No. 17 issued by the Ministry of Labour of the Government of India.[22] Fifteen volunteers from Indian textile mills were tested under conditions that duplicated as nearly as possible those used at Ft. Knox.[12, 13] The responses of acclimatized Indian workmen to thermal stress were very nearly identical to the responses of the acclimatized American soldiers who volunteered as subjects at Ft. Knox. However, these Indian workmen were observed under experimental conditions rather than under the usual conditions of their work and had been carefully acclimatized prior to the tests. It would be desirable to compare the data obtained elsewhere on men at work at their normal employment, with the data obtained under controlled laboratory conditions. There are, however, very few observations reported from industry that include, for example, data on dry-bulb and wet-bulb temperatures along with a description of the physiologic effects on workers. In connection with his analysis of 244 cases of collapse among miners in the Kolar Gold Fields of Mysore State in India, Caplan[8] recorded dry-bulb and wet-bulb temperatures which were measured in the underground areas where these

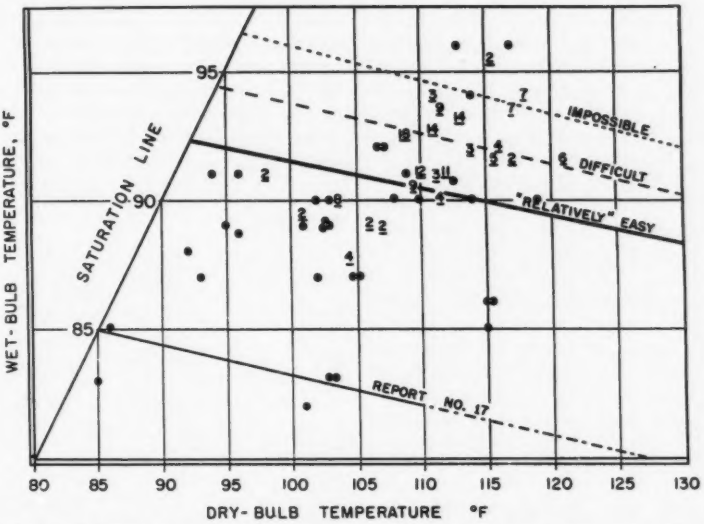

FIGURE 1. Upper limits of thermal stress observed among human experimental subjects compared with reported mild cases of heat-induced collapse among miners (Caplan). (Numbers indicate multiple cases at indicated conditions.)

men worked. These data have been plotted on graphs, see Figures 1 and 2, and, to serve as a basis for comparison, the curves representing data obtained at Ft. Knox have been inscribed on these same graphs.

All of the 194 cases classified by Caplan as mild had almost completely recovered when admitted to the hospital.[8] Histories revealed the occurrence of fatigue, giddiness, nausea and, less frequently, vomiting, cramps and loss of consciousness; the latter persisted no more than 10 minutes whenever it occurred. Also observed were hypotension, diminished pulse pressure, bradycardia and mild dehydration. Mild cases were kept in bed 12 to 36 hours and put on a fluid diet. When plotted in Figure 1 according to the dry-bulb/wet-bulb temperatures prevailing at the points in the mines where these cases occurred, most of the cases are located above the "Ft. Knox" line that is labelled "RELATIVELY" EASY. However, 63 cases are located below this line. In this connection it is interesting to note that Caplan reported that 65 among the group of 194 mild cases were suffering from a febrile illness. These 65 cases are not identified so they need not be the ones that are shown below the line labelled "RELATIVELY" EASY, but febrile illness of some of these persons could provide a clue as to why they collapsed under conditions of thermal stress that were not especially severe.

Caplan describes the moderate and severe cases, of which there were 50, as presenting a clinical picture resembling surgical shock.[8] The least severe of these cases represented a transition stage between mild and moderate. The criteria for severe cases were (a) a state of complete unconsciousness and (b) the absence of a palpable pulse at the wrist. For both moderate and severe cases the prodromal symptoms included fatigue, asthenia, giddiness and nausea always followed by vomiting, cramps and unconsciousness. The skin was cold and clammy, the temperature was subnormal (95° to 96°F.). The patients exhibited all the signs of shock

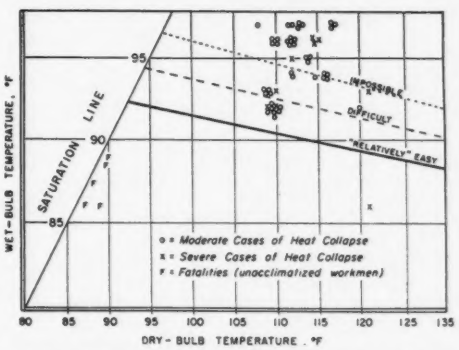

FIGURE 2. Upper limits of thermal stress observed among human experimental subjects compared with reported cases of moderate and severe heat-induced collapse (Caplan) and heat-induced fatalities among miners.

and all were treated on the general principles for shock. Fluids, including salt water (one teaspoon NaCl to one pint water), were administered extensively, orally to all conscious patients, augmented with rectal administration if vomiting persisted, and administered intravenously to all comatose or pulseless patients. Plotted in Figure 2 in terms of the dry-bulb/wet-bulb temperatures that prevailed in the work areas where these men collapsed, it can be seen that 34 cases are located at or near the "Ft. Knox" line labelled IMPOSSIBLE. The one "severe" case in Figure 2 that is located below the line labelled "RELATIVELY" EASY may represent a person found by Caplan to have been febrile because of illness unrelated to the thermal stress in the mines. Two of the severe cases were found to be suffering from upper respiratory infections and four of these severe cases had lost acclimatization due to an absence of several days away from their work.

In Figure 2, one fatality reported by Dreosti[10, 11] and four fatalities reported by Mavrogordato and Pirow[22] have been designated by the letter **F**, located on the graph at the points representing the dry-bulb/wet-bulb temperatures that were observed where the fatal exposures to heat occurred. These persons had not been properly acclimatized for work in the hot areas where they collapsed. In connection with one fatality Dreosti reported the incident as being related to unauthorized transfer of this workman to a particularly hot area of the mine.

In connection with the average dry-bulb/wet-bulb temperatures reported outside the steel mill where Collings and coworkers[9] observed 491 "heat cases" (mostly heat exhaustion), their data have not been plotted on these graphs because the temperatures in the areas where the men worked were not reported. However, 30 of the cases occurred at a time when the outside temperatures were just below the line of temperatures labelled "RELATIVELY" EASY, see Figure 1. However, it seems reasonable to assume that the conditions inside the mill were a little if not a great deal more severe than was indicated that particular day by the prevailing temperatures outside, 99°F. with 80 per cent relatively humidity.

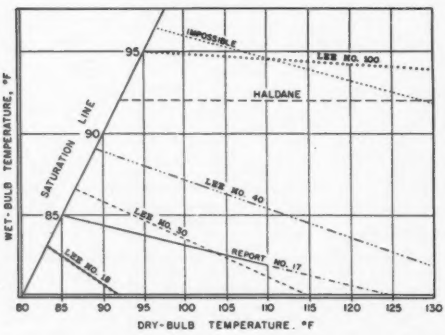

FIGURE 3. A comparison of several suggested upper limits of thermal stress for man.

Discussion

One part of Report No. 17 is devoted to developing a recommended upper limit of thermal stress that may be safely tolerated by workmen.[22] This recommended level, shown in Figure 3, was defined by the dry-bulb/wet-bulb temperatures 85°/85°F. and 110°/82°F. Since the principle application of this upper limit was likely to be made in Indian textile mills, no dry-bulb temperatures above 110°F. were included because it was believed that it was both desirable and feasible to keep temperatures below but certainly no higher than 110°F.

The exact slope of the line that can be accepted as properly representing the safe upper limit of thermal stress for man has not been discovered. The line chosen by Haldane[17, 18] would be the line of the wet-bulb at 92°F., see Figure 3. At those high levels that are likely to produce collapse, the wet-bulb temperature is very important and may well be all important. At lower temperatures, however, the dry-bulb temperature assumes progressively more importance. In an analysis of many factors concerned with the effects of thermal stress on man, Lee[21] has devised a formula that (a) gives for high temperatures, the most consideration to wet-bulb temperatures (in the neighborhood of 95°F.) and (b) for cool conditions, the most consideration to dry-bulb temperatures (in the neighborhood of 50°F.). His formula gives a progressive change in emphasis to dry-bulb and wet-bulb temperatures for points between 50°F. and 95°F. and on a psychrometric chart the slopes of lines representing thermal strain have, therefore, a progressive change in slope. At a wet-bulb temperature of 95°F. a line proposed by Lee is stated to be associated with severe thermal strain which produces distress and/or collapse very quickly among non-acclimatized persons and to this line he assigns the number 100. This line is just above and parallel to the line suggested by Haldane. Another line designated by Lee by the number 40, see Figure 3, represents the temperatures that will cause "distress in 2 hours, failure in 5 hours". Its slope is slightly away from the adjacent wet-bulb lines and toward the dry-bulb lines. Another line designated by Lee as number 30 corresponds

fairly well with the upper limit of thermal stress suggested in Report No. 17, except that it gives a little more emphasis to the dry-bulb temperature and a little less emphasis to the wet-bulb temperature. Lee defines this level of thermal strain as that at which deterioration in mental performance begins. Still another line, number 18 shown in Figure 3, represents the upper limit of thermal strain recommended by Lee for daily work.[21]

An upper limit of thermal stress may be properly based on the responses to heat among unacclimatized persons if these persons are to live and work in the temperate zones of the world. Thermal stress in those areas usually occurs only at infrequent intervals of time which are more-or-less unpredictable and are dependent upon prevailing climatic conditions. For those conditions the upper limits of thermal stress suggested by Belding and Hatch[5] and by Lee[21] may be entirely appropriate for workmen.

In other circumstances where acclimatization is urgently necessary either because of climatic conditions or by the nature of certain industrial processes, or both, the upper limit of thermal stress for workmen may properly be somewhat higher as indicated in Report No. 17,[22] see Figure 3. This limit is below the levels at which all of the cases of moderate and severe heat collapse have been reported and below the levels at which nearly all reported cases of mild heat collapse occurred. In fact, for persons in good health who have physiologically young circulatory systems and who are fully acclimatized the limit might be safely set as high as the line designated in the accompanying figures as "RELATIVELY" EASY. The line representing a lower level of thermal stress was chosen for the upper safe limit for workmen, however, because it was apparent that among persons suffering form temporary illness such as the common cold, from general ill health or malnutrition, and/or from chronic diseases, collapse may be induced by levels of thermal stress somewhat less severe than the "RELATIVELY" EASY level. It cannot be stressed too frequently, however, that acclimatization must be given careful attention and efforts must be made to regain it after acclimatization has been lost. In Africa[10, 11, 19, 20, 28, 29] and in India[8] it has been quite apparent that acclimatization is, to some extent, lost whenever workmen are absent from work for a few days or a few weeks during which they experience only temperatures that are below those at which they are required to work. When they returned to be subjected again to elevated levels of thermal stress, reacclimatization was exceedingly important.

The fact that Moss[24, 25] reported that only heat cramps had been observed in connection with the range of dry-bulb/wet-bulb temperatures, 98°–102°F./85°–87°F., appears to give additional corroboration to the upper limit suggested here. If plotted on a psychrometric chart the zone of thermal stress referred to by Moss would be located below the "RELATIVELY" EASY line but above the line suggested in Report No. 17 as the upper safe limit for workmen.[22]

In the reports of a number of experimental and carefully controlled investigations there have been several suggestions for upper limits of thermal stress. Plotted as lines on a psychrometric chart these several limits would not coincide exactly. However, the amounts of clothing worn during the investigations varied from one investigation to another as did the amounts of work performed, the velocity of air movement, etc. When these variables are taken into consideration it seems likely that these results are all in fairly good agreement. Application of the formula of Lee to the several groups of data may clarify some of the seeming differences. It would be well to point out here the fact that a minimum of clothing was worn by the subjects in the investigations at Ft. Knox and at Ahmedabad, India. Also, very scant attire was worn by the workmen in the mines at Mysore and the mines in South Africa. These particular groups of data are sufficiently comparable to warrant a fair degree of confidence in setting the safe upper limit of thermal stress for these workmen.

For the purpose of this discussion, the problem of radiant heat from very hot surfaces in industry has been intentionally omitted. This topic merits a separate discussion at some later time when more in-plant data and experimental observations become available. Haines and Hatch[16] have been making extensive observations on this phase of the problem. At this time it is safe to say that exposures to high levels of radiant heat will push down the safe upper limits of ambient air temperatures unless workmen are adequately shielded from the sources of radiant heat.

Summary

There is a relatively large body of information relating to experimental investigations that describe the physiologic responses of man to severe thermal stress. However, collapse is the one response or endpoint that can be most consistently predicted for persons in good health. The dry-bulb/wet-bulb temperatures related to this response have been well established with human experimental subjects.

Far less information is available which de-

scribes in a similar way the occupational exposures to thermal stress. The data for which related dry-bulb and wet-bulb temperatures have been reported, have been compared on graphs on which certain experimental data had also been plotted. These data give sufficiently good agreement to permit the selection of an upper safe limit of thermal stress for workmen.

A line described by the dry-bulb/wet-bulb temperatures, 85°F./85°F. and 110°F./82°F., had been suggested as the upper safe limit of thermal stress for acclimatized workmen in Indian textile mills. The location of that line on a psychrometric chart appears to have been a reasonably good choice and gives a relatively wide margin of safety which will help avoid heat casualties among acclimatized workmen who become ill for reasons other than exposure to heat.

A somewhat lower limit may be desirable for unacclimatized workmen.

This analysis of data was made in part in connection with preparation of the subject matter of Report No. 17 which describes investigations that were conducted by Mr. N. S. Mankiker, Chief Adviser Factories of the Indian Ministry of Labour and Employment in collaboration with the Technical Cooperation Mission to India, a part of the Foreign Aid Program of the United States of America.

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Determination of Ozone in Air by Neutral and Alkaline Iodide Procedures*

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The determination of concentrations of ozone in air in the range of a few parts per million has become an increasingly important problem as a result of current toxicological and air pollution studies. Toxic concentrations of ozone may be encountered in high altitude flights, as well as in some rocket work and in inert gas shielded arc welding. Ozone also appears to play a key role in certain types of smog forming processes, as well as in the generation of eye irritants and plant-damaging substances. Although a great many methods for ozone determinations have been employed over a period of years[6, 10], the uncertain stoichiometry and the lack of specificity continue to be serious problems. The iodide chemical methods appeared to be among the most promising, and were therefore selected for investigation.

Many workers have used iodimetric methods for the determination of concentrations of ozone in the range of several per cent by volume and higher. They have investigated the stoichiometry by comparison of the amounts of iodine liberated with the amounts of ozone determined by physical measurements of gas density or pressure change. Thus Lechner[5] found that both neutral and alkaline (0.2 *N* KOH) potassium iodide (0.2 *M*) absorbed ozone efficiently and yielded the same amount of iodine, equivalent to one oxygen atom in the ozone molecule. This stoichiometry was confirmed by Boelter et al.[2] for 4 to 20% ozone by weight absorbed in 0.2 *M* potassium iodide buffered to various pH values from 2.3 to 12.3. In another recent study by Birdsall et al.[1], similar results were obtained for 1 to 25 mole-per cent ozone (1.7 to 41 wt.%) absorbed in 2% unbuffered potassium iodide, but high results were obtained with boric acid buffered solutions. The discrepancy between Birdsall's findings and those of Boelter was explained by the difference in sampling equipment: Boelter obtained good results with acid solutions because he used an absorption bulb in which the surface reactions could cause local alkalinity at the point at which ozone was absorbed. Birdsall used a bubbler in which absorption proceeded at the acid pH of the bulk of the solution. Although there are also contrary reports in the literature, the use of both neutral and alkaline iodide solutions for high concentration ozone analysis seems to be established.

Similar procedures have been applied to the determination of low concentrations of ozone in the parts per million range. Renzetti and Romanovsky[6] reported that, when sampling Los Angeles air, the neutral method gives apparently low results (perhaps due to reducing pollutants) in the early and late hours of the day, and high results (perhaps due to other oxidizing pollutants) during the peak smog time of day, both as compared to ultraviolet spectrophotometric determinations of ozone. Smith and Diamond[9] recommended a reagent consisting of 1% potassium iodide in 1 *N* sodium hydroxide, acidified after sampling with ⅓-volume of 36% phosphoric acid to release the iodine for spectrophotometric estimation. This was modified by Byers, Saltzman, and Hyslop[3], who found that the interference from nitrogen dioxide could be greatly reduced, and the colors stabilized, if the phosphoric acid were saturated with sulfamic acid, which destroys nitrite. The specificity of the iodide reaction was investigated by Effenberger[4], who used 0.01 *N* potassium iodide buffered to various pH values. The amounts of iodine liberated by ozone varied from 90% at pH 9 to 113% at pH 1 as compared to that at pH 7. For nitrogen dioxide the variation in amounts of iodine released was much greater, the corresponding figures being 70% and 340%. Thus the two could be distinguished by simultaneous analyses at two different pH values. The 1% potassium iodide in 1 *N* sodium hydroxide reagent was thought to be more nearly specific than the neutral reagents in the presence of oxidized hexene by Saltzman[7], who also reported 62 to 70% stoichiometry for 3 to 27 ppm (v/v) ozone (as compared to the iodine released in a neutral reagent). The validity of the application of neutral and alkaline iodide reagents to the

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determination of low concentrations of ozone was therefore investigated.

The preparation of known low concentrations of ozone has always been a major difficulty in this type of investigation. A flow system was set up in which a stream of ozone from a dielectric type ozonizer could be diluted with air purified by scrubbing with dichromate in concentrated sulfuric acid followed by calcium chloride and silica gel. Flows were measured with rotometers and could be adjusted to give known dilution ratios. The reagents were used for wide ranges of undiluted and diluted ozone concentrations, and samples of widely varying size were collected. The assumption was made that the high concentration analyses were likely to be correct, and the deviations resulting from varying the sample sizes and from the dilution were studied.

Ozone Procedures

Reagents:

Three reagents were tested extensively: Reagent I, 20% potassium iodide in 0.1 *M* KH_2PO_4–0.1 *M* Na_2HPO_4; reagent II, 1% potassium iodide in the same neutral phosphate buffer medium; and reagent III, 1% potassium iodide in 1 *N* sodium hydroxide, acidified after sampling with ⅕-volume of 36% phosphoric acid saturated with sulfamic acid. Reagent I was prepared fresh and used within several hours. Reagents II and III were allowed to stand for several days before being used. This stabilized the blank. With each of the three reagents it was necessary to determine the reagent blank and to deduct it from all standardizations and determinations. Only the highest grade analytical reagents were used. Reagent I is commonly used in oxidant recorders[6], but was found too unstable and photosensitive for the usual manual procedures. Reagent II was found to yield almost as much iodine as reagent I with much superior stability. Reagent III proved the most suitable for field studies where hours or days might elapse between collecting and analyzing the samples.

Sampling:

Samples were collected in 10 ml of reagent in midget impingers using air-flow rates of 1 to 3 liters per minute. Other simple bubblers may be used; however, the midget impinger is convenient and effective. Sampling of high concentrations by means of evacuated flasks was tried and found to be unsuitable.

Measurements:

Iodine liberated by the ozone was measured photometrically or titrimetrically. The iodine absorbancy at 352 millimicrons wavelength was measured by a Beckman DU Spectrophotometer. Test tubes of 2-centimeter light path and matched to 0.5% transmission were used in a special holder. Distilled water was used in the reference tube.

Titrations were made with 0.005 *N* sodium thiosulfate in a semi-micro buret and using a visual endpoint with starch indicator.

Reagents I and II, Procedures and Standardization:

After collection, the samples were transferred immediately to photometer tubes and the absorbancies determined.

Standard graphs of absorbancy vs. iodine (or ozone) were plotted from readings from a series of prepared standards. Standard 0.0100 *N* iodine solution was freshly diluted with reagent solution to various strengths from zero to 0.00004 *N* (5.08 micrograms I_2 per ml) and the absorbancies determined. The ozone equivalent was calculated on the basis of $O_3 \simeq I_2$ (1 ml 0.01 *N* $I_2 \simeq$ 240 micrograms O_3).

Reagent III, Procedure and Standardization:

The phosphoric acid reagent for use with the alkaline reagent was prepared as follows: Several grams of reagent quality sulfamic acid were dissolved in 150 ml warm distilled water, then 126 ml of 90% H_3PO_4 were added and the mixture was made to 300 ml volume with distilled water. After cooling, the precipitated sulfamic acid was removed by decantation (or centrifugation) and saved for preparation of future batches of reagent.

Samples in 10 ml of the alkaline iodide reagent were rapidly acidified and mixed with 2.0 ml of phosphoric acid reagent. The mixture (in a stoppered container) was cooled to room temperature in a water bath, and the absorbancy determined 5 to 10 minutes after acidification.

A strong stock solution of potassium iodate (0.2973 gram per liter) was diluted 1:10 with distilled water to give a dilute standard of which 1 ml was equivalent to 105.8 micrograms I_2. Aliquots of 0.1 to 0.5 ml of this dilute standard were diluted to 10 ml with reagent III, acidified and the absorbancies read. Calculations were originally made on the basis of 3 $O_3 \simeq KIO_3$ (1 ml dilute stock $KIO_3 \simeq$ 20 micrograms O_3). Our work, as will be discussed, showed the need for a correction factor of 1.54 which would make 1 ml dilute stock $KIO_3 \simeq$ 30.8 micrograms O_3.

Calculations:

The following relationship may be used rather

than graphs for calculation of ozone concentrations:

$$\text{ppm } O_3 = K\frac{A}{V}$$

Where A is the absorbancy corrected for the blank, V is the volume in liters of air sampled (corrected to 25°C and 760 mm-Hg), and K is the standardization factor. For our two centimeter tubes we found K to be 4.61 for reagent I, 4.88 for reagent II, and 9.13 for reagent III. The last figure is higher because it incorporates the correction factor previously mentioned and because the corresponding absorbancies are lowered by the dilution of the sample to 12 ml by the acidification.

Effects of Sample Size

The undiluted ozone concentrations ranged from 300 to 5000 ppm, depending upon the flow rate and oxygen content of the gas passing through the ozonator, and the voltage applied. The photometric determination of these levels using reagents I and III had previously[7] been found unsatisfactory because of the extreme dilutions required to permit readings in the photometer and of a non-linear relationship between the amounts of iodine and sample sizes as shown in Figures 1 and 2. These figures give the data obtained by simultaneously sampling with calibrated evacuated bottles of various sizes, containing these reagents. The amounts of iodine were estimated photometrically, and some of the higher amounts were checked by titration. The plots show the total absorbance (the measured absorbance, corrected for the blank, multiplied by the dilution factor) for 2-cm cells versus the sample size. It can be seen in Figure 1 that the neutral method with 20% KI (reagent I) gave curved lines in the useful photometric region, which approached straight lines only at absorbances requiring extreme dilutions. The values indicated for ozone on the figure were assigned on the basis of the limiting slopes of the curves at high values. The alkaline method (reagent III), Figure 2, gave almost straight lines, which seemed to converge to a negative absorbance at zero sample volume. This negative absorbance intercept may be regarded as the "ozone demand" of the reagent, and was found for both evacuated bottle and impinger samples at high concentrations. The "ozone demand" appeared to increase with the age of the reagent, and was reduced somewhat by the addition of iodate to the sampling reagent to produce artificially an appreciable positive iodine blank. This "ozone demand" was fairly constant through a small range of ozone concen-

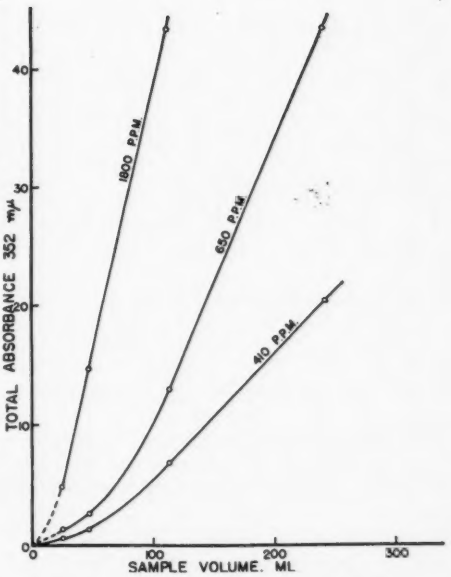

FIGURE 1. Effect of sample size on photometric determinations of high concentrations of ozone with neutral 20% KI reagent.

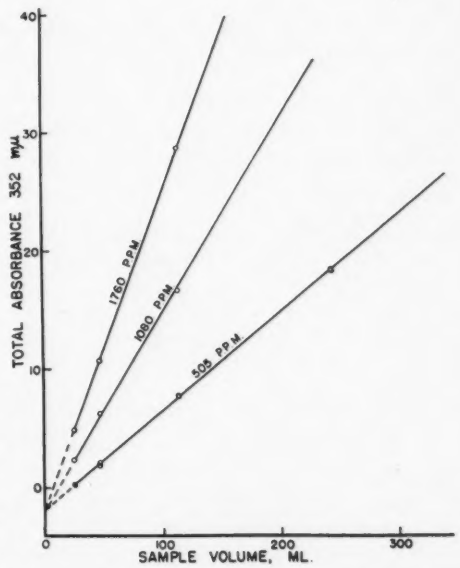

FIGURE 2. Effect of sample size on photometric determinations of high concentrations of ozone with alkaline KI reagent.

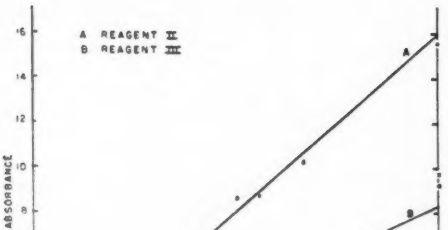

FIGURE 3. Effect of sample size on photometric determinations of low concentration (4.25 ppm) of ozone with neutral (A) and alkaline (B) 1% KI reagents.

trations, but increased somewhat at much higher absorbances and ozone concentrations. The reasons for these discrepancies are not known.

Subsequently analyses of high concentrations of ozone were carried out by titrimetric procedures. Effects of errors due to sample size were thus minimized by liberation of larger amounts of iodine.

Photometric analyses of low concentrations of ozone with reagents II and III were employed successfully since only slight discrepancies were found to result from variations of sample size. This is shown in Figure 3, which also shows a difference in stoichiometry to be discussed later. The midget impinger with 10 ml of reagent was used at sampling flow rates of 1, 2 and 3 liters of air per minute. The differences in flow rates had little or no effect except as they determined the volume sampled. Both reagents show good linear relationships of absorbance and sample size for ozone concentrations in the 0.1 to 20 ppm (v/v) range. The results by the alkaline procedure are consistently lower with a tendency to an increasing proportion of iodine for very large samples.

TABLE I

Simultaneous Analyses of Undiluted and Diluted Ozone Concentrations by Reagents II and III (Neutral and Alkaline)

Reagent II		Reagent III	
Undiluted (ppm)	Diluted 1/100 (ppm)	Undiluted (ppm)	Diluted 1/100 (ppm)
5350		4760	
5050		5400	
4310		4250	
3510		4360	
3030	25.1	3045	14.4
1895	15.9	1650	11.4
1413	14.4	1262	9.72
1280	11.5	1125	8.19
980	8.13	808	4.06
791	6.70	636	3.67
547	4.92	600	3.69
473	3.56	379	2.73
453	3.78	394	2.56
439		374	
437		375	
433		384	
425	3.81	353	2.53

Effects of Ozone Concentration

A comparison of analyses with reagents II and III was made at both high and low concentrations. Low concentrations were obtained by accurately diluting high concentration ozone 1:100 with purified air. Simultaneous samples were collected and analyzed as previously discussed with the results given in Table I. From these data it can be seen that the two methods give fair agreement at concentrations of several thousand ppm but that they diverge at lower concentrations, with the results by the alkaline procedure being consistently lower. Measurements of the diluted ozone by the neutral reagent gave about 85% of the calculated value with little variation, while the alkaline reagent gave much lower and more erratic results. Inasmuch as paired samples were collected simultaneously the differences could not be due to fluctuations in the ozone concentrations. Subsequent tests revealed a 6% loss of ozone in the system between the point of sampling the high concentration and the point of dilution. It appears that the stoichiometry of reagent II is more nearly constant with such changes of concentration.

Relative Values by Neutral and Alkaline Reagents

In Figure 4 the relationship between analyses by the neutral and alkaline reagents I, II and III is shown for corresponding samples collected simultaneously or at very close intervals over a wide range of ozone concentrations. Because of the extreme range of concentrations a log-log plot is used. Concentrations above 100 ppm (v/v) were determined titrimetrically and below this, photometrically.

The differences between the alkaline and neutral analyses cannot be explained by differences in absorption efficiencies of sampling since these were found to be high with all three

reagents. When two impingers were used in series the second never showed more than a few per cent of the amount of iodine released in the first impinger. Very little ozone was found to be destroyed by the water, the alkali or the sampling apparatus. When 1 *N* sodium hydroxide (reagent III without KI) was used in the first of two impingers in series, 85 to 90% of the ozone passed into the second impinger. The apparently lost ozone was found to be dissolved in the alkali, and by the addition of KI and acid it would release iodine for its determination. Thus it seemed likely that the absence of iodine in a second sampler was significant, and that the absorption efficiencies were high.

Differences likewise cannot be explained on the basis of standardization. The neutral reagents were standardized by adding dilute iodine solutions, and the alkaline reagent by adding standard potassium iodate. The iodine extinction coefficients in units of absorption (to logarithmic base 10) per cm per gram per liter were as follows: (I) 104, (II) 99, (III) 97. The slightly higher coefficient for reagent I may be explained by the higher iodide concentration, which intensifies the iodine color. The slightly low coefficient for reagent III may be due to losses of iodine in the acidification procedure.

Assuming that the relationship in Figure 4 would be a straight line, or nearly so, the best fit was determined by the method of least squares. The photometrically determined points gave a line with a slope of 1.016, and all points shown gave a line with a slope of 1.045. Calculated relationships of results at several concentrations according to the best fit of photometric determinations are:

ppm O_3	0.01	0.1	1.0	10.	100.
% alk/neut.	60%	62%	64%	66%	69%

Applying the data for both photometric and titrimetric results, we find the corresponding values by alkaline determinations to range from 50 to 80% of the neutral reagent results.

The scatter of results shown by Figure 4 is such that the relationship of alkaline to neutral analyses of 65%, as indicated by the line A/N = 0.65 can be applied over the range of 0.01 to 30 ppm and perhaps beyond at either end while staying within the probable experimental error. Since the neutral analyses are presumed to be more nearly correct, results of alkaline analyses should be multiplied by a correction factor of 1.54.

Stoichiometry

It thus seems evident that the actual reaction between the ozone and the iodide must have a different stoichiometry in alkaline solution. The reaction between ozone and iodide is commonly given as:

(1) $O_3 + 2H^+ + 2I^- \rightarrow O_2 + H_2O + I_2$

In weak alkali, the equivalent reactions for the same stoichiometry are commonly given as:

(2) $3\ O_3 + I^- \rightarrow 3\ O_2 + IO_3^-$

followed upon acidification by:

(3) $IO_3^- + 6\ H^+ + 5\ I^- \rightarrow 3\ I_2 + 3\ H_2O$

We found experimental evidence to indicate that in strong alkali this pattern is not followed. When portions of samples in reagent III were acidified to pH 6.2 with solid boric acid, the iodine released was approximately 50% of that resulting from the usual acidification to pH 2. No iodine was obtained from the reagent III with added iodate, upon acidification with boric acid. With added periodate, such acidification yielded 14 to 20% of the iodine obtained at pH 2. This compares with 25% reported for periodate by Willard and Merritt.[11] A reasonable explanation of these data would appear to be the formation of hypoiodite by the following reaction:

(4) $I^- + O_3 \rightarrow IO^- + O_2$

The dismutation of hypoiodite to give iodate appears to occur over a period of many hours. Another possible explanation could be the formation of iodite.

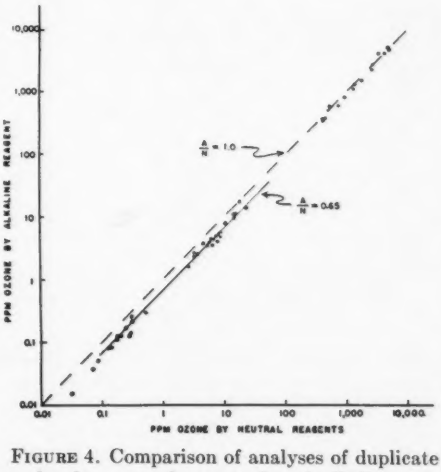

FIGURE 4. Comparison of analyses of duplicate samples for ozone by neutral and alkaline reagents. A/N is the ratio of alkaline to neutral values for analyses of pairs of duplicate samples. The dashed line (A/N = 1) is the theoretical line for identical stoichiometry by both methods.

The stoichiometry is being confirmed in an independent way by gas titrations. A stream of ozone is mixed with either nitric oxide, or nitrogen dioxide, and enough flow time is allowed for complete reaction after which the mixture is analyzed for ozone and nitrogen dioxide. In the former case, the ozone is converted to equivalent amount of nitrogen dioxide:

$$(5) \qquad O_3 + NO \rightarrow O_2 + NO_2$$

In the latter case, the following reaction occurs:

$$(6) \qquad O_3 + 2\,NO_2 \rightarrow O_2 + N_2O_5$$

Since reaction (5) is 260 times as fast as (6), in the presence of excess nitric oxide, no appreciable quantities of nitrogen pentoxide need be expected. Linear relationships are being found between the amounts of ozone and the amounts of nitrogen dioxide generated or consumed; the latter being determined with Saltzman reagent.[8] These systems appear to change very sluggishly when the reactant concentrations are changed, perhaps because of surface effects, and the systems are not yet completely satisfactory.

The data for one run in which nitrogen dioxide was titrated with ozone are given in Figure 5. A dynamic system was used in which the gases were mixed at high concentrations, allowed to react, then diluted into a large chamber with purified air. The nitrogen dioxide input was held constant and the ozone input was varied.

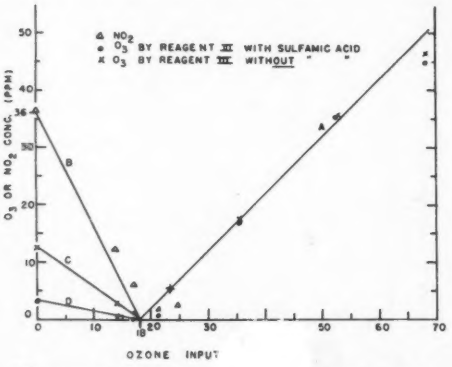

FIGURE 5. Plot of dynamic gas phase titration of NO_2 with O_3. NO_2 input held constant at 36 ppm as O_3 input was increased.

Line A—linear increase of excess O_3.

Line B—linear decrease of NO_2 with addition of O_3.

Line C—interference of NO_2 to give false O_3 results with reagent III without sulfamic acid.

Line D—interference of NO_2 to give false O_3 results with reagent III with sulfamic acid.

The ozone input to the chamber was calculated from calibrating runs without nitrogen dioxide. Ozone was determined with reagent III, corrected for stoichiometry by the factor of 1.54. Ozone analyses to the left of the end point (line D) represent the interference of excess nitrogen dioxide (or nitrogen pentoxide) with the analytical method. The line C shows the much higher interference of NO_2 when sulfamic acid is not added to the phosphoric acid reagent. A sharp end point is not obtained, but can be extrapolated with approximately the theoretical stoichiometry. Line B shows the consumption of nitrogen dioxide, and line A shows the accumulation of excess ozone.

Comparison of Procedures

Either neutral or alkaline iodide procedures may be applied for the determination of ozone in air, the latter procedure requiring the use of a correction factor for stoichiometry.

One of the advantages of the alkaline procedure was found to be the relative stability of the exposed reagent prior to acidification. This would permit collection of samples in the field, and completion of the analyses in the laboratory. In one test, aliquots of a pooled alkaline sample were acidified and read at intervals for three days after sampling. Most of the loss of 10 to 20% which was observed occurred in the first day, so that analysis of even older samples was practicable. Some lots of sampling reagent behaved better than others in this respect. A special reagent in which the sodium hydroxide was ozonized, then boiled before addition of potassium iodide did not give significantly better results.

The reproducibility of both methods for replicate samples of ozone was not as precise as could be expected from the experimental conditions. Reagent II seemed to be the best in this regard. It showed maximum differences up to 5%, while reagent III showed maximum differences up to 10%, and average differences of about 5%. Apparently the variations with reagent III occurred during the ozone sampling step since much closer agreement was obtained by acidifying aliquots of a large pooled sample.

Reagent III was found to require rapid acidification in order to avoid low yields of iodine. Upon very slow acidification of reagent III, with either ozone or iodate added, such losses have been observed. Similar losses occur upon addition of standard iodine to the alkaline reagent before acidification and regardless of the rate of acidification, but there was no loss when iodine was added after acidification. Apparently in the former cases iodine is released at the surface of acid drops and is absorbed into the surrounding alkali

where the loss occurs. These errors are easily avoided by rapid acidification of samples as evidenced by the good agreement of the previously mentioned iodine extinction coefficient for reagent III with expected values.

A limited amount of work has been carried out to determine the effect of interfering pollutants. Sulfur dioxide decreased the amount of iodine liberated from all reagents, the difference between reagents II and III being small. Nitrogen dioxide yielded 8 to 11% interference by method III, and about the same by II. Five tests with 500 ppm of nitric acid vapor showed an average interference of 0.5 ppm ozone by method III.

Interferences from other smog constituents have not been completely evaluated. Comparative analyses by the two methods of natural and synthetic (ozone-hexene) smogs appear to give a different pattern of results than for ozone-air mixtures. In some cases, analyses by the alkaline procedure gave results higher than those by the neutral procedure.

Summary

One per cent KI in neutral buffered or alkali solutions was found to be more stable and useful than 20% KI, in bubblers for the collection and analysis of ozone in air. Either of the former may be used to determine low concentrations of ozone; however, there is a difference in their stoichiometry. Over the range of 0.01 to 30 ppm (v/v) results by the alkaline procedure should be multiplied by 1.54 to correct for stoichiometry. The neutral reagent has the advantages of not requiring the extra step of acidification and of an apparently more nearly uniform stoichiometry. The alkaline procedure is preferable when final analysis may be delayed for any considerable period. The results of analyses of smog mixtures by either method cannot be regarded as specific for ozone, and their significance is not clear.

Experiments with boric acid for acidification of samples in the alkaline reagent show that some mechanism other than oxidation of iodide to iodate or periodate is involved in this procedure, such as possibly formation of hypoiodite.

Data from preliminary experiments with gas phase titrations of nitrogen dioxide and nitric oxide against ozone appear to confirm the stoichiometry of the neutral reagent to be one mole of iodine released for each mole of ozone.

Acknowledgment

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Health Hazards and Control of an Epoxy Resin Operation

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THE EPOXY resins are strong and resilient plastics that have interested engineers since they first appeared in 1950. Thermosetting and heat-resistant, these plastics when cast and heat-cured are especially useful in applications requiring resistance to shock and vibration. They are also—unfortunately—somewhat toxic, since tertiary and tetra-amines released during the uncured, or wet, state cause allergic reactions in almost all personnel exposed. The epoxy resins are therefore an industrial hygiene problem of some importance; a problem that, unless non-toxic hardeners are developed, can be expected to increase as these resins come into wider and wider use. An excellent paper discussing experimental data and chemical characteristics of many of the epoxy resins appears in the February issue of the *A.M.A. Archives of Industrial Health.*

When epoxy resins were first investigated by Sandia Corporation, the ordnance contractor to the Atomic Energy Commission, nothing was known of their toxic effects. The first indication of toxicity occurred in January 1951 and appeared as an inflammation of the eyes and forearms of a plastics shop employee. In the procedure used at that time, the plastic was poured over fiberglas formed on a solid mold and, if necessary, was smoothed with the hand; the procedure had been used for a number of years with polyester resins with only occasional cases of dermatitis, primarily because of carelessness while handling cleaning solvents. The inflammation was therefore diagnosed at first as no more than a contact condition, and the employee was temporarily withdrawn from work on plastics. Recurrence of the condition when the employee returned, even though he cooperated fully in avoiding contact, was the first suggestion that epoxy resins were hyperallergens and thus an industrial hygiene problem.

Plastics shop personnel, on this indication, were then advised to follow a precautionary program such as is used in radiochemistry laboratories, that is: (1) put on clean coveralls each morning and observe extreme personal cleanliness; (2) keep hands out of the solvents being used to clean up tools or spills (contact with plastic solvents is believed to be responsible for most of the allergies); (3) wash hands with soap and water if uncured material is touched; (4) take showers at the end of the shift; (5) cover the floor or work benches with heavy butcher paper wherever the uncured material is being mixed or handled; (6) wear rubber gloves during all wet layup operations; and (7) do not smoke or eat in the area where layups are being made.

Alleviation of the problem was also sought through improvements to ventilation in the plastics shop, which at the time was small and crowded. However, the industrial hygiene division found that since exhaust air was largely overbalanced by supplied air, eddying could not be prevented and thus a draft could not be maintained away from workers. Because work on epoxy resins was thought to be exploratory only and probably temporary, approval could not be obtained for a program to improve ventilation and other working conditions.

That personal hygiene and care in handling were not sufficient became clear as one after the other of the plastics shop personnel became sensitized. Although some improvements were made to the ventilation and personnel cooperated fully, the allergies continued—not by direct contacts but by fumes of the amines given off by the wet plastic. The allergies also tended to cause a sensitization to the formerly innocuous polyester resins.

As the epoxy resins received greater attention from development engineers and the "temporary" work continued, almost two-thirds of the personnel became sensitized. Drastic action was precipitated finally when an order for some very large shapes was received. The industrial hygiene division, after reviewing the procedure required, closed the shop until adequate space and ventilation could be provided.

After management was informed of the past situation and the situation that could be expected if operations continued without improvement, approval for a new area with much more space and much better facilities was obtained. Setting up the new area afforded an opportunity to plan operations with industrial hygiene requirements fully in mind. Since not all the ducting and

FIGURE 1. Plastics shop—dry area

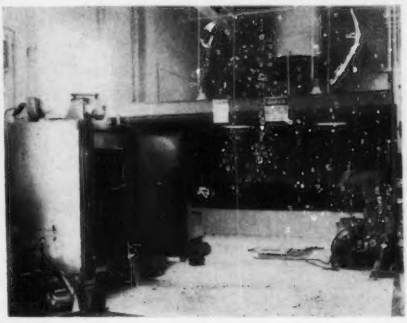

FIGURE 2. Plastics shop—wet layup area

exhaust facilities desired were commercially available, some of the design work was also done by industrial hygienists.

One of the principal design considerations was separation of operations in which fumes are evolved from those in which the dry plastics only were handled. This design was undertaken for two reasons: (1) uncured material could be confined to areas where special exhaust and handling equipment was avaliable, and (2) the area where dry material only was handled would provide an area in which personnel who had become sensitized could still work. This arrangement permitted the retention of several workers who would otherwise have had to be transferred.

Figures 1 and 2 show the new plastics shop. The area in which dry operations are carried out is shown in Figure 1. Figure 2 shows the waterwall and bake-out oven in the area where fumes may be evolved which we call the "wet layup" area.

The exhaust system has a total capacity of about 60,000 cubic feet per minute. An input system with a capacity of 50,000 cubic feet per minute provides makeup air to replace that exhausted. This makeup air is supplied through the overhead ducts visible in the two figures. One change to the exhaust system was made

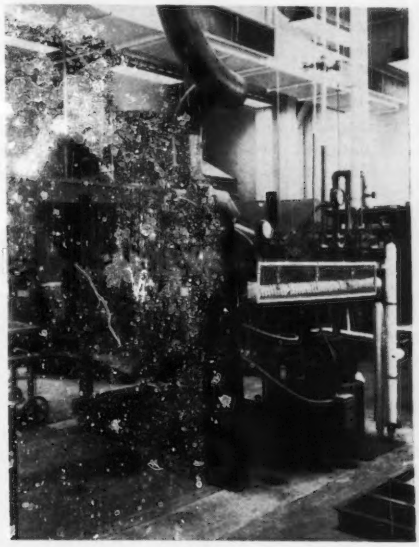

FIGURE 3. Fifty-ton press

FIGURE 4. Down-draft table

after these photographs were taken. With exhaust blowers located, as shown, near the input to the duct, duct pressure was greater than room pressure and any leakage in ductwork resulted in pumping of amine fumes back into the shop over a period of time. Blowers have therefore been located near the outlet so that negative duct pressure is maintained.

The major feature of the area in which wet plastics are handled is the water-wall in Figure 2. This area is 15 feet deep from the water tank to the outer edge of the hood. Exhaust capacity is 24,000 cubic feet per minute. All mixing and wet layup are done in this area. Workers in this area wear surgical gloves taped to their coverall sleeves to ensure that hands and arms do not come in contact with the plastic; goggles and safety glasses need not be worn. When there is no possibility of splashing of solvent or plastic, wet layup may be carried out without these safety devices, which are required elsewhere in Sandia Corporation on all machining and chemical operations. This procedure was begun after it was observed that inflammation and irritation from epoxy resins was worse when these safety devices were worn, apparently because of the concentration of fumes behind the safety glasses or face shields.

Since resin spills are an important source of inadvertent contacts, cleanliness in the area is essential. In the water-wall area not only is butcher paper used under the mold but the floor covering, a plastic film, is scraped off and replaced once a month.

Figure 3 shows a 50-ton press in which some curing operations are carried out and which therefore requires an exhaust system. Since the top bed of the press moves up and down, flexible ductwork was required. The press had to be further modified by the addition of sheet-metal panels to restrict the flow of air to the immediate area around the plastic.

Figure 4 shows a down-draft table with a rotating top. In addition to the flow of air through the top grate, air may be drawn across the work when the square panel in the front of the unit is removed. Baffles are provided in this outlet so that the down-draft flow is not stopped when the panel is removed.

After the improvements were completed, no cases of sensitization occurred in the plastics shop. The few cases that have occurred at Sandia Corporation were the result either of carelessness in handling or of working in areas that were not approved.

In summary, the experience of Sandia Corporation in its work with the epoxy resins suggests the following recommendations:

(1) High standards of both personal and work-area cleanliness must be maintained. The work area must be kept free of spills; when spills do occur, they must be cleaned up immediately.

(2) Personnel should be made well aware of effects of the material with which they are working. The responsibility to ensure that personnel are educated in the characteristics of the material will belong to both the industrial hygienist and the plastics shop supervisor.

(3) Both the industrial hygienist and the supervisor should be able to recognize the early symptoms of sensitization. Personnel with these symptoms should report immediately to the industrial physician, since early diagnosis and treatment are important.

(4) In a large plant or laboratory, personnel outside the plastics shop must be made aware by industrial hygienists of the potential hazards of the epoxy resins and warned not to begin small operations with these plastics without adequate safeguards. In Sandia Corporation this information and other industrial hygiene information is distributed plant-wide in "Industrial Hygiene Bulletins."

Although proper industrial hygiene practices come close to eliminating the epoxy resins as a health hazard, an even more hopeful approach is the substitution of low-toxicity aliphatic amines for the toxic agents. In a recent article[2] Ingberman, Walton, Pitt, and Paul indicate that the hydroxyalkyl hardeners do not cause sensitizations. Progress in this area may sometime reduce or eliminate the problem. Until that time, however, the epoxy resins must continue to warrant the attention of the industrial hygienist.

References

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HYGIENIC GUIDE SERIES

Chlorine Dioxide

I. Hygienic Standards

A. RECOMMENDED MAXIMUM ATMOSPHERIC CONCENTRATION (8 hours): 0.1 part of vapor per million parts of air, by volume (ppm).[1] One part per million has also been recommended.[2] Further industrial experience and medical observations are necessary for confirmation.

(1) Basis for Recommendation: Animal studies and limited observations in industry.[1, 2, 4]

B. SEVERITY OF HAZARDS:

(1) *Health*: High for both acute and chronic exposures. Chlorine dioxide is a strong respiratory and eye irritant. Acute exposures by inhalation cause bronchitis and pulmonary edema. Symptoms can be delayed and the recovery may be slow. Deaths have occurred.[2] Symptoms observed in affected workers are coughing, wheezing, respiratory distress, nasal discharge, eye and throat irritation. These probably result from repeated acute exposures. Repeated exposures may cause chronic bronchitis.[4]

(2) *Fire and Explosion:* High. Explosive when concentration in air exceeds 10 per cent.[1] Explosion risk is increased when chlorine dioxide contacts organic materials. Explosion and fire may be caused by sparks at 20°C and 36 mm partial pressure of chlorine dioxide.[6] Will detonate if heated rapidly to 100°C.

C. SHORT EXPOSURE TOLERANCE: Repeated exposures of rats to about 10 ppm for four hours daily resulted in deaths; whereas, exposure to approximately 0.1 ppm, five hours daily for 10 weeks, had no detectable effect.[1] Animals survived two-hour exposures to 20 ppm, though some species exhibited symptoms.[4] Elkins states that 5 ppm is irritating and two cases of poisoning (1 fatal) resulted from exposure to less than 19 ppm.[2]

D. ATMOSPHERIC CONCENTRATION IMMEDIATELY HAZARDOUS TO LIFE: Delayed deaths occur in animals after single exposures to 150–200 ppm for less than one hour.[4]

II. Significant Properties

Chlorine dioxide is a greenish-yellow irritant gas at room temperature. It decomposes in sunlight. It is usually generated from sodium chlorite at the point of use.

Chemical formula:	ClO_2
Molecular weight:	67.5
Specific gravity:	3.09 (liquid at 11°C)
Relative vapor density:	2.4 (air = 1)
Boiling point:	9.9°C at 730 mm Hg
Melting point:	−59°C
At 25°C and 760 mm Hg,	
1 ppm of vapor:	0.00276 mg/liter
1 mg/liter of vapor:	363 ppm

III. Industrial Hygiene Practice

A. RECOGNITION: Used as a bleaching agent in the wood pulp, paper, textile, and food industries and for odor control in water treatment. Chlorine dioxide is liberated from acidic solutions of sodium chlorite or from reactions of sulfur dioxide with sodium chlorate in acid solutions. The gas is greenish-yellow with an unpleasant, characteristic, ozone-like odor. Odor threshold is uncertain, but is above 0.1 ppm.

B. EVALUATION OF EXPOSURES:

(1) *Direct instrumentation:* None.

(2) *Chemical method:* Sampling and analysis of concentrations in air can be done by the method of Giertz, as described by Dalhamn.[1]

C. RECOMMENDED CONTROL PROCEDURES: Maintain atmospheric concentrations below 0.1 ppm. This will usually require some process ventilation and enclosure. Evolution of chlorine dioxide from aqueous solutions can be reduced by the addition

of sodium sulfite.[3] Particular attention is required to prevent leaks and short peak exposures. Respirators, safety showers and eye washing fountains should be available. Appropriate fire and explosion precautions should be taken.[5]

IV. Specific Procedures

A. First aid: Remove from exposure. Keep at rest until seen by a physician. For eye or skin contact wash with copious amounts of water.

B. Special medical procedures:

(1) *Preplacement:* Individuals with pre-existing pulmonary disease or with significant heart disease should not be exposed.

(2) *Periodic examination:* Evaluate health of exposed persons, especially for symptoms related to acute exposures, chronic bronchitis, or disturbances in pulmonary physiology.

(3) *Treatment of acute exposures:* Persons with acute exposures require complete bed rest and careful observation for development of pulmonary edema, which may be delayed. 100% oxygen should be used as indicated.

V. Literature References

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3. Evans, J. C. W.: *Paper Trade Journal,* p. 44 (Feb. 18, 1957).
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Phosphoric Anhydride

Phosphorus Pentoxide

I. Hygienic Standards

A. Recommended maximum atmospheric concentration (8 hours): Exposures should be kept below the level at which discomfort occurs. It is believed a level of 1.0 milligram of P_2O_5 per cubic meter of air (mg/m^3) will accomplish this.[6]

(1) *Basis for Recommendation:* Human experience in industry.[6]

B. Severity of hazards:[1, 2, 3, 5]

(1) *Health:* Moderate for both acute and chronic exposures. Phosphoric anhydride is a local irritant and a very strong dehydrating agent. With moisture it forms phosphoric acid which is corrosive to the skin, mucous membranes, and the eyes. The acid formed is not considered as hazardous as nitric or sulfuric acid. Very high concentrations of the anhydride will cause violent coughing, although those exposed regularly to the industrial fume apparently become acclimated to some degree. No evidence of systemic poisoning from either acute or chronic exposures.

(2) *Fire:* None; however, the heat evolved by the reaction with water is sufficient to ignite flammable or combustible materials.

C. Short exposure tolerance: Data from one study[6] indicate 10 mg/m^3 will cause coughing, while 50–100 mg/m^3 in unacclimated individuals is intolerable. Those accustomed to exposure apparently endure higher concentrations, perhaps up to 100 mg/m^3, without difficulty.

D. Atmospheric concentrations immediately hazardous to life: Unknown; however, it is not believed that a person would, unless trapped, tolerate concentrations that might be immediately hazardous to life.

II. Significant Properties

A fluffy, practically white, crystalline powder. It may be present as a fume at some processes. It has a very slight phosphorus-like odor. The commercial grade analyzes at least 99.5%.

Chemical formula:	P_2O_5
Molecular weight:	142
Specific gravity:	2.38 (Technical grade)
Melting point:	347°C (657°F)

Solubility: Reacts readily with moisture or water, with evolution of heat. With water the reaction is violent.

III. Industrial Hygiene Practice

A. RECOGNITION: By its irritant effects.

B. EVALUATION OF EXPOSURES:

(1) *Direct instrumentation:* None available.

(2) *Chemical method:* Absorption in alkali followed by a molybdenum blue method, using ferrous sulfate instead of stannous chloride as the reducing agent.[4, 6] P_2O_5 is difficult to collect, so the use of alkali solution in a jet type impinger must be followed by a filter paper, such as No. 41 Whatman, to trap particles passing through the impinger.

C. RECOMMENDED CONTROL PROCEDURES: Maintain workroom atmospheres below 1.0 mg/m³ by means of process enclosure and/or local exhaust ventilation. Avoid skin and eye contact. For very dusty conditions, the use of full face industrial canister masks, coveralls, rubber gloves, aprons, and foot protection are indicated.

IV. Specific Procedures

A. FIRST AID: The dust should be washed off the skin with large quantities of soap and water and if exposure was severe, this flushing should be continued for 15 minutes. Eyes should be washed with large quantities of water for at least 15 minutes and medical attention should be secured at once. For irritation from inhalation of dust, remove from further exposure and call a physician at once. Gargling with water may relieve throat irritation.

B. SPECIAL MEDICAL PROCEDURES (including preplacement): None.

V. Literature References

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2. FLEMING, A. J., et al.: *Modern Occupational Medicine*. Lea and Febiger. Phila. (1954).
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6. STOKINGER, H. E., AND RUSHING, D. E. (U. S. Public Health Service): Personal communication.

Because of space limitations, it is impossible to list all methods of exposure evaluation. The selections have been made on the basis of current usage, reliability, and applicability to the usual industrial type of exposure. Any specific evaluation and/or control problem will involve professional judgment. This can best be done by professional industrial hygiene personnel.

Respiratory protective devices are commercially available. Their use, however, should be confined to emergency or intermittent exposures and not relied upon as primary means of hazard control.

A relative scale is used for rating the severity of hazards: nil, low, moderate, high, and extra hazardous.

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———Methyl Ethel Ketone
———Methylene Dichloride
———Nickel Carbonyl
———Nitrogen Dioxide
———Ozone
———Pentachlorophenol and Sodium Pentachlorophenate
———Phosphoric Acid
———Silica (Free Silica, Silicon Dioxide)
———Sulfur Dioxide
———Sulfuric Acid
———Toluene
———Trichloroethylene
———1,1,1,—Trichloroethane
———Vanadium Pentoxide
———Xylene
———Zinc Oxide